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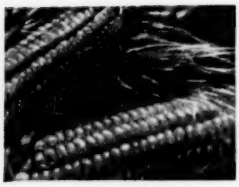
The Vital Story of CORN ENRICHMENT
(CORN MEAL—CORN GRITS)

by Science Writer



Since Pilgrim days corn has been a popular food in American diets. Today dishes using corn meal or corn grits are served at breakfast, lunch, dinner.

As scientific information was gathered about this popular grain, it was found that Nature had been bountiful with certain food essentials and stinting with others. Unfortunately, only small amounts of certain vitamins and other nutrients which are essential to human health were found to be present. Furthermore, unavoidable losses occurred when the grain was degermed. Degerming allows the product to be packaged for nationwide distribution without spoiling.

When corn millers realized this situation, they took an important step to correct it. They used enrichment.[1]

Enrichment is essentially a simple process. It restores important vitamins and minerals to whole grain values. And, in the case of whole corn meal, which is naturally low in niacin and iron, it increases the values for these essentials over whole grain levels.

These are the vitamins used in enriching corn meal and corn grits:

Thiamine, also called vitamin B_1 which helps to build physical and mental health. It is essential for normal appetite, intestinal activity and sound nerves.

Riboflavin, also called vitamin B_2. This vitamin is essential for growth. It helps to keep body tissues healthy and to maintain proper function of the eyes.

Niacin, another "B" vitamin, is needed for healthy body tissues. Its use in the American diet has done much to make a serious disease called pellagra disappear.

In addition to the "B" vitamins, *iron* is used in all enrichment of corn meal and grits. And, at the miller's option, calcium and vitamin D may be added.

Our Federal government and many state governments have established maximum and minimum enrichment levels which the various forms of corn meal and grits must meet to be acceptable in commerce. For instance, the Federal Standards for enriched corn meal and enriched corn grits are as follows:

	MIN.	MAX.
	(Mg. per lb.)	
Thiamine	2.0	3.0
Riboflavin	1.2	1.8
Niacin	16.0	24.0
Iron	13.0	26.0

ADDITIONAL AT MILLER'S OPTION

Calcium	500.0	750.0
Vitamin D*	250.0†	1,000.0†

*Although an optional ingredient, not considered necessary in corn enrichment. †U.S.P. units per lb.

The Federal Standards further provide that the levels for enriched grits must not fall below 85% of the minimum figures after a specific rinsing test.

The enriching ingredients are combined into a form called premix which millers add during processing so that enrichment is spread evenly throughout the product.

The vitamins used in enrichment are manufactured. Scientists first learned the chemical composition of a vitamin, then isolated the pure substance. Then they "built" a duplicate. This duplicate is identical with Nature's product chemically and in biological activity. A vitamin is a vitamin regardless of its source, just as salt is salt whether it comes from a mine or is evaporated from the sea. So efficient is large scale manufacturing that vitamins are available to millers at a lower cost than if they were extracted from natural sources.

The Hoffmann-La Roche people, pioneers in research and manufacture, make top-quality vitamins literally by the tons. In fact, a large percent of the vitamins used in enrichment of corn meal and grits, family white flour, white bread, macaroni products, and white rice, come from the Roche plant. Using amazingly complex processes with scientific production controls, Roche employs modern, special equipment which fills whole buildings, each one a city block square and many stories high.

This article, reprints of which are available without charge, is published as a service to millers of corn meals and corn grits by the Vitamin Division of Hoffmann-La Roche Inc., Nutley 10, New Jersey. In Canada: Hoffmann-La Roche Ltd., 286 St. Paul Street, West, Montreal, Quebec.

[1]An excellent color film on the enrichment of corn meal and grits has been produced by Clemson Agricultural College, Clemson, S. C. Widely recognized as an outstanding educational film on food and nutrition it is being shown by many health departments and schools.

Millers of corn meal and corn grits help in the fight against dietary deficiency diseases and in promoting good nutrition when they enrich their products with thiamine, riboflavin, niacin and iron plus calcium.

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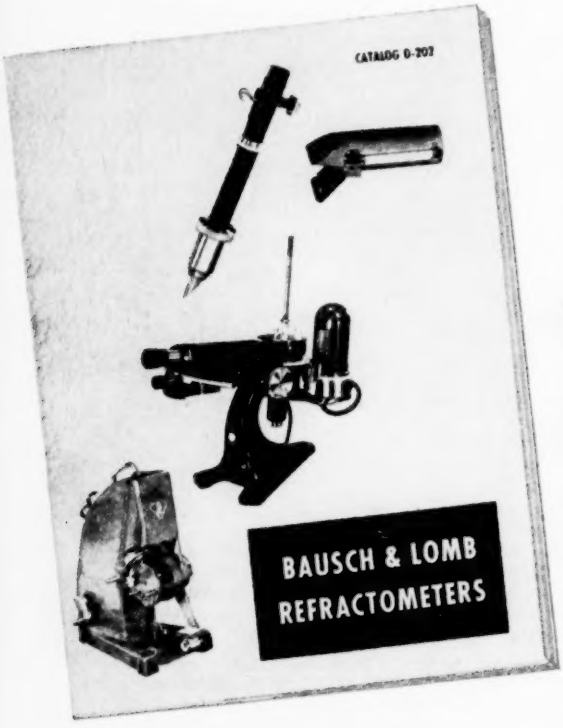

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HEAT PROCESSING OF BEEF. III. OBJECTIVE MEASUREMENT OF CHANGES IN TENDERNESS, DRAINED JUICE, AND STERILIZING VALUE DURING HEAT PROCESSING[a, b]

R. G. TISCHER,[c] H. HURWICZ, AND J. A. ZOELLNER
Iowa State College, Ames, Iowa

(Manuscript received February 28, 1953)

Bard and Tischer *(1)*, working with Canner and Cutter grade beef shoulder clods found that definite relationships existed between the times and temperatures used in processing and the objective qualities measured. In each case, the change in a quality attribute was found to increase with increased processing temperature. The tenderness of the beef, measured as shear force, was found to decrease at an increasing rate as the temperature of processing was raised from 225 to 255°F. Correspondingly, the rate of increase in drained juice was found to increase over the same range of processing temperatures.

In this work, only 3 processing temperatures were used and as a result, no detailed information was gained concerning the functional relationships involving changing processing temperature.

The objective of the present work was to investigate changes in tenderness and in the accumulation of drained juice from beef during canning over a relatively wide range of processing times and temperatures, and finally, to discover the mathematical relationships among these variables.

As no theoretical relationship was available pertaining to the variables involved, as in Parts I and II of this series *(2, 3)*, it was found necessary to develop one solely on the basis of experimental data. In this way an attempt was made to maintain throughout this series of investigations a consistent approach—a method concerned primarily with the fundamental laws governing the physical changes in beef occurring during heat processing, expressed in terms of mathematical relationships.

EXPERIMENTAL PROCEDURE

The raw product was obtained from matched sets of beef rounds (inside, outside, and knuckle) which were used at the rate of one set per week.[d]

The experimental design is shown in Table I. This design takes account of the week of operation, the time of processing, the cut of meat, and the temperature of processing. It should be noted that the design has been randomized by rows over retort

[a] Journal Paper No. J-2267 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 1123.

[b] This paper reports research undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces, and has been assigned No. 416 in the series of papers approved for publication. The views or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsement of the Dept. of the Army.

[c] Present address: Qm. Food and Container Institute for the Armed Forces, Chicago, Illinois.

[d] The matched sets of round cuts of beef used in this work were supplied through the kind cooperation of Armour & Company, Chicago, Illinois.

TABLE 1

Experimental design used in tracing the changes in tenderness, drained juice and sterilizing value of beef during heat processing

Week	Sets of time values[a]	Temperature level[f]		
		Cut I	Cut II	Cut III
1	1	5 6	1 2	3 4
2	2	1 2	3 4	5 6
3	3	3 4	5 6	1 2
4	4	6 4	5 1	3 2
5	5	5 1	3 2	6 4
6	6	3 2	6 4	5 1
7	7	1 6	2 4	3 5
8	8	3 5	1 6	2 4
9	9	2 4	3 5	1 6
10	10	2 5	3 6	1 4
11	11	1 4	2 5	3 6
12	12	3 6	1 4	2 5
13	1	1 3	4 5	2 6
14	2	4 5	2 6	1 3
15	3	2 6	1 3	4 5
16	4	2 3	4 6	1 5
17	5	1 5	2 3	4 6
18	6	4 6	1 5	2 3
19	7	1 3	4 5	2 6
20	8	4 5	2 6	1 3
21	9	2 6	1 3	4 5
22	10	2 5	3 6	1 4
23	11	1 4	2 5	3 6
24	12	3 6	1 4	2 5
25	1	3 5	1 6	2 4
26	2	1 6	2 4	3 5
27	3	2 4	3 5	1 6
28	4	5 6	1 2	3 4
29	5	3 4	5 6	1 2
30	6	1 2	3 4	5 6
31	7	1 4	2 5	3 6
32	8	3 6	1 4	2 5
33	9	2 5	3 6	1 4
34	10	1 5	2 3	4 6
35	11	2 3	4 6	1 5
36	12	4 6	1 5	2 3

[a] Actual time values are shown in Table 2.
[f] Where temperature level numbers correspond with those shown in Table 2.

temperatures. A complete set of retort temperatures is found in random order in each row of the design. This was done to enhance the information gained from a consideration of all 3 cuts together and at the expense of information concerning separate cuts.

The time set values referred to in Table 1 refer to the processing times listed in Table 2. It should be noted that these times are different for each temperature. These values were arrived at by setting up a series of 12 processing times at 225°F., based on previous beef processing data and a similar set at 315°F. and interpolating between for the remaining processing temperatures by graphical means as shown in Figure 1.

Measurements of tenderness were made using a Bratzler-Warner shear force machine. The samples used were ½-inch cylinders cut from both the raw and the processed beef at the rate of 10 cylinders per 300 × 308 can.

Drained juice values were obtained from 300 × 308 cans packed with approximately equal quantities of beef by draining the juice from the can after processing and measuring the weight of the juice after 2 minutes of draining.

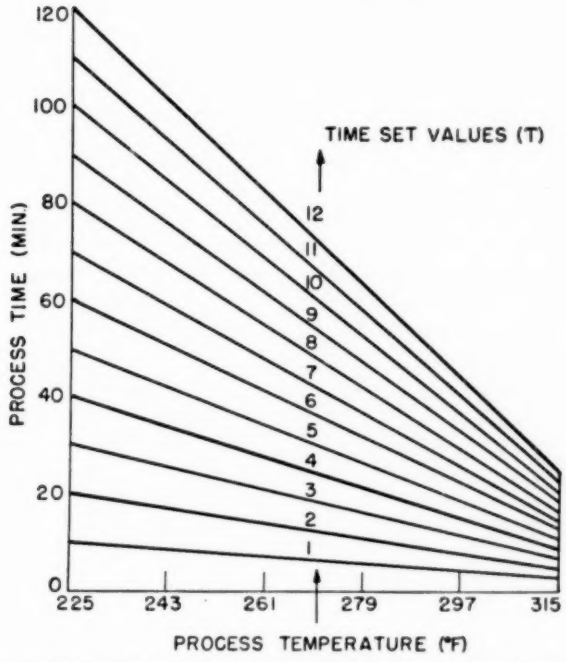

Figure 1. Relationship among time and temperature of processing and time set values used in the experimental design.

TABLE 2

Schedule of retort temperatures and processing times

Temperature	1	2	3	4	5	6
RT	225°F.	243°F.	261°F.	279°F.	297°F.	315°F.
Time set values	Time (min.)	Time (min.)	Time (min.)	Time (min.)	Time (min.)	Time (min.)
1	10	8	7	5	3	2
2	20	17	14	10	7	4
3	30	25	21	16	11	6
4	40	34	27	21	14	8
5	50	42	34	26	18	10
6	60	50	41	31	21	12
7	70	59	48	36	25	14
8	80	67	54	42	29	16
9	90	76	61	47	32	18
10	100	84	68	52	36	20
11	110	93	75	57	40	22
12	120	101	82	62	43	24

RESULTS AND DISCUSSION

During the operation of the design used for measuring the effect of processing temperature for sets of times during processing of beef, measurements of shear force were made at the rate of 10 determinations for each can processed and means of these values are shown in Table 3 for each time value and each processing temperature. The mean values for the raw meat were Cut I = 7.51 lbs., Cut II = 13.00 lbs., and Cut III = 8.64 lbs. indicating that Cut II was much less tender than Cuts I and III.

TABLE 3

Mean value of shear force (tenderness) of processed beef

Time set value	Processing temperature					
	225°F.	243°F.	261°F.	279°F.	297°F.	315°F.
1	11.2	10.4	10.8	10.1	10.9	10.7
2	12.3	13.9	16.8	16.4	13.2	10.7
3	11.7	11.6	13.8	13.3	12.9	10.0
4	12.5	12.4	15.3	12.2	10.9	16.3
5	11.7	8.8	7.3	8.7	9.6	11.2
6	11.4	8.8	8.4	10.1	11.3	11.0
7	11.4	4.4	5.0	3.7	6.4	9.7
8	4.3	5.1	6.9	3.6	5.6	9.4
9	5.8	6.4	4.1	3.3	8.5	6.5
10	6.4	4.9	5.9	4.1	5.0	5.0
11	7.4	5.0	4.4	3.8	5.0	5.9
12	6.7	4.0	6.8	3.7	3.9	8.6

The mean values of shear force for processed beef averaged over cuts in every case start with a relatively small value, increase to a maximum and then fall off gradually as time goes on, this trend apparently continuing at a more rapid rate as the processing temperature increases. The general form here observed, in addition to the fact that considerable variability appears to be inherent in the data, led to the analysis of this information by means of relatively simple regression techniques in which only approximately linear portions of the curves were fitted by regressions.

TABLE 4

Linear regressions for the linear portion of the shear force curves, by temperatures

Temperature	Equation
°F.	
225	$Y = -.067X + 13.74$
243	$Y = -.118X + 14.61$
261	$Y = -.166X + 16.51$
279	$Y = -.254X + 16.86$
297	$Y = -.263X + 14.97$
315	$Y = -.517X + 17.53$

X = process time. Y = mean shear force averaged over cuts.

This curve-fitting technique yielded the linear regression equations shown in Table 4. The values of β are shown in Table 5 and the estimated values, $\hat{\beta}$ and values of $\hat{a}$ are shown in the same table. Observations of

the β values derived from this technique indicated that they were related exponentially and as a result a regression was run to determine the coefficients of this exponential regression among processing temperatures.

The equation $\hat{\beta}=0.04431\times10^{-0.00\,994\,(T-200)}$ gives the relationship among the β's shown in Table 5 on the assumption that this is an exponential function. Figure 2 shows the exponential relationship between the slopes of the shear force lines and the processing temperature. The estimated β's or $\hat{\beta}$ values shown in the third column of Table 5 were derived through use of this equation and indicate fairly good agreement throughout the temperature range. The intercept values shown in the table are all higher than would be expected because of the fact that the linear approximation fails in the vicinity of zero time and at that point the relationship is a curved function which was only estimated graphically.

TABLE 5

Regression coefficient of time-shear force curves for six processing temperatures[a] and estimated β from the equation $\hat{\beta}=a^{e^{-KT}}$

Process temperature (T)	β	$\hat{\beta}$	$\hat{a}$	n
°F.				
225	−.067	−.074	13.74	12
243	−.118	−.108	14.61	11
261	−.166	−.157	16.51	11
279	−.255	−.228	16.86	11
297	−.263	−.330	14.97	11
315	−.517	−.580	17.53	9

where $\hat{\beta}=0.04431\cdot10^{-0.00994\ (T-200)}$

[a] Only the linear portion of the curves was used for calculation; this accounts for the high values of a.

The exponential prediction equation may be used to indicate the expected rate of change of shear force at any processing temperature between 225°F. and 315°F. for approximately the conditions which obtained during this experiment.

In an effort to show these relationships more graphically, a three-dimensional graph was made and is presented in Fig. 3. For this purpose, the original values of the β's taken from Table 5 were used to plot the slopes of lines at the various temperatures, then the lines shown on the graph were drawn from the data. The lines on the graph numbered 4 through 13 are lines of equal shear force at any temperature. The lines numbered 20 through 120 are lines of equal time. Through use of this graph an idea may be obtained of the relationship among the variables investigated and it should be possible to estimate approximately, lines of time, temperature, and shear force from this graphic presentation.

Another point should be noted carefully. Although in the graph no errors are shown, it is apparent from a consideration of the data that the surface pictured in the graph should actually have a thickness attributable to error since the errors or at least the variations among readings persist through all of the calculations and should be taken account of in any predictions which are made.

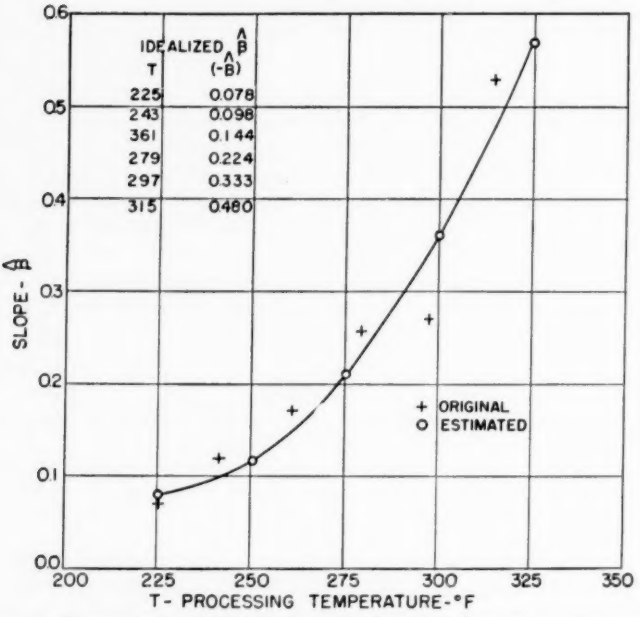

Figure 2. Exponential regression of slopes of shear force lines over processing temperatures.

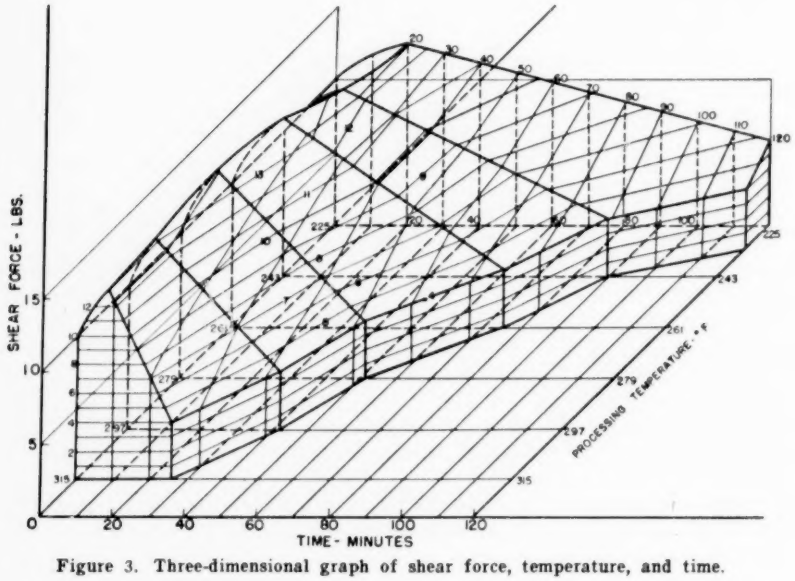

Figure 3. Three-dimensional graph of shear force, temperature, and time.

The problem of characterizing the three-dimensional surface defined by time, temperature, and shear force with regard to simplification within the confines of the experimental error was also examined in another way.

The arrangement of times and temperatures (Table 2 and Figure 1) suggests the possibility that within any given time set value[b] the shear force may be constant over processing temperatures. If this simplification can be justified, the general solution can be easily obtained.

In order to establish the preceding point an analysis of variance was made within each time set value. The experimental designs within these time set values are known as incomplete block designs, where the blocks are represented by the cut and the plots by the halves of the cuts to which the treatments were applied (see Table 1).

The analysis for each time set value has the form:

	d.f.
Blocks ignoring treatments	8
Treatments eliminating blocks	5
Error	4

Before pooling the 12 analyses it was noted that in only one out of the 12 time set values did the usual F-test of significance, based on 5 and 4 degrees of freedom, indicate significant differences at the 5% level. Upon pooling the sum of squares for treatments and error from all 12 experiments the F-test based on 60 and 45 degrees of freedom was found to be not significant on the basis of a 5% significance level.

Taking account of this result the data were pooled to obtain shear force as a function of time set value. Table 6 below gives the estimated shear

TABLE 6

Estimated shear force for each time set value (pooled data)

Time set value	Estimated shear force
1	10.69
2	13.73
3	12.77
4	13.27
5	9.65
6	10.31
7	6.68
8	5.82
9	5.67
10	5.13
11	5.27
12	5.44

force at each time set value and Fig. 4 is a plot of estimated shear force against the time set value.

The dotted line in Figure 4 represents an idealized curve which is

[b] Time set values are the numbers 1-12, each one representing a set of 6 times corresponding to the six processing temperatures as shown in Table 2.

suggested by the data. It can be assumed that a general function exists such that:

$$\tau = h\,(T)$$

where

τ = estimated shear force
T = time set value.

The function h (T) can be approximated by the usual regression techniques involving the use of polynomials or orthogonal polynomials.

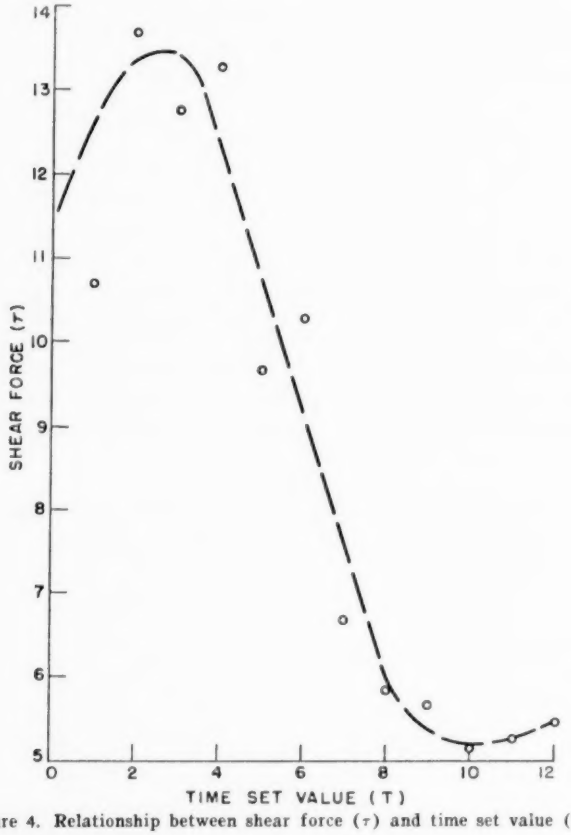

Figure 4. Relationship between shear force (τ) and time set value (T).

Assuming that h (T) can be satisfactorily obtained, a question arises as to whether τ can be expressed as a function of time and temperature. It may be observed from Table 2 and Figure 1 that except for rounding:

$$y = 10T - \frac{8T}{5}x$$

where

y = time in minutes

x = processing temperature coded in the following way:

$$x = \frac{225^\circ - T}{18^\circ}$$

where

$$225^\circ \leq T \leq 315^\circ$$

and

T = processing temperature (uncoded)

making the substitution in $h(T)$:

$$\tau = h\left(\frac{y}{10 - \frac{8}{5}x}\right)$$

which generates a three-dimensional surface similar to that shown in Figure 3.

If attention is restricted to the linear portion of the curve, say from time value 3 through time value 8, $h(T)$ is approximated by:

$$\tau = \alpha + \beta T.$$

Employing the usual regression techniques to estimate α and β it was found that:

$$\tau = 18.21 - 1.5389\,T$$
$$\pm .2741$$

Where the number under the estimated regression coefficient is an estimate of its standard error. The regression is significant beyond the 1% level.

Now since

$$\tau = h\left(\frac{y}{10 - \frac{8}{5}x}\right),$$

we have by substitution:

$$\tau = 18.21 - 1.5389\left(\frac{y}{10 - \frac{8}{5}x}\right).$$

If a regression coefficient β is defined for a fixed temperature then:

$$\beta = \left(\frac{-1.5389}{10 - \frac{8}{5}x}\right).$$

A table of β's for the six temperatures used in the experiment appears in Table 7.

TABLE 7

Regression coefficients and their standard errors for six processing temperatures

Processing temperature	β	Standard error of β
°F.		
225	−.1539	.0274
243	−.1832	.0326
261	−.2263	.0403
279	−.2959	.0527
297	−.4275	.0762
315	−.7694	.1370

Comparison of the β values of Table 7 with the β values in Table 5 indicates that the relationship to processing temperature is exponential in each case. The actual slope values differ in magnitude because of the differences inherent in the two methods used to produce them.

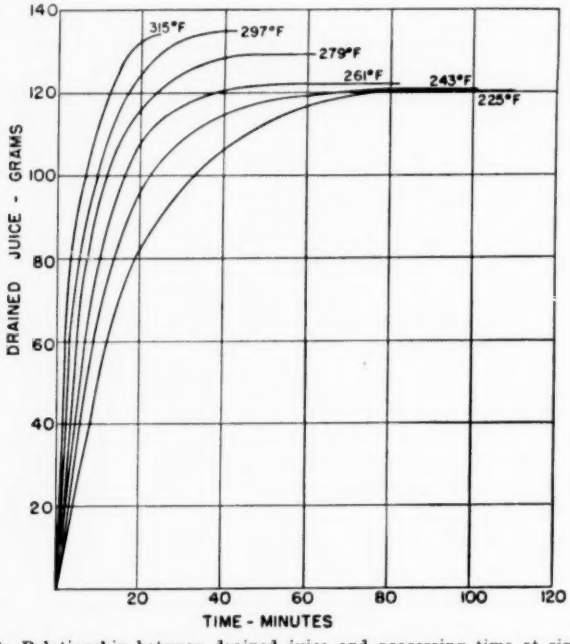

Figure 5. Relationship between drained juice and processing time at six processing temperatures.

The drained juice resulting from processing at the temperatures already noted and throughout the series of times in the design used yielded a set of values for drained juice at the end of each process. These values

are tabulated in Table 8 and indicate that accumulation of drained juice in a can under the conditions imposed appears to be a reasonably well behaved function. As shown in Figure 5 this function is usually monotonic non-decreasing and definitely related to the processing temperature.

A clearer picture is presented in Figure 6 in which drained juice is related to processing temperature and time. The lines of equal drained juice and equal time are found to be almost linear and may be used to interpolate on the surface to indicate the length of time necessary at any processing temperature to produce any quantity of drained juice contained on the surface.

In the evaluation of these data several choices were available for functions to fit the curves. One obvious one would have been the use of orthogonal polynomials, however, this procedure requires considerable labor and as a result, a transformation was used in an attempt to linearize these relationships. The transformation $t^{1/3} \times T^2 \times 10^{-3}$ yielded data which appeared to be roughly linearly related to the drained juice.

In view of the concordant results obtained with this transformation, these data were treated as a linear regression to obtain a relationship for time and temperature with drained juice, as simply as possible. This treatment yielded a linear estimate of the slope and an estimate of the intercept in the following equation:

$$Y = 0.33\,X + 38.02, \text{ where}$$

$\beta = 0.33$

$a = 38.02$

$X = t^{1/3}\,T^2\,10^{-3}$

$Y =$ drained juice in grams

$t =$ time in minutes

$T =$ temperature degrees F.

Through use of this prediction equation it should be possible to estimate the drained juice which would be found in any processing time-temperature combination used in this experiment. The standard deviation from regression was calculated as

$$\text{Standard Deviation from Regression} = \sqrt{\frac{\Sigma y^2 - (\Sigma xy)^2}{\Sigma x^2}}{n-2},$$

where the x and y are deviations from their respective means. This treatment yielded the result ± 9.17 as the deviation in drained juice for the linear regression.

Algebraic expansion of the linear regression in terms of the transform yields the equation,

$$Y = \frac{t^{1/3}T^2}{3030} + 38.02.$$

In this space, no simple estimate of error exists and if the equation is used in this form, other methods would have to be found to estimate the errors involved.

TABLE 8

Drained juice (grams) accumulated after processing at six temperatures for twelve times

Time set value	Processing temperature					
	225°F.	243 °F.	261°F.	279°F.	297°F.	315°F.
1	49.1	54.3	66.7	75.4	67.1	87.1
2	82.3	90.0	86.9	88.9	84.8	78.6
3	90.1	100.0	111.0	111.4	107.4	95.8
4	112.4	114.4	117.4	118.4	114.0	106.5
5	109.4	116.2	115.1	114.0	113.2	110.5
6	112.5	113.8	118.7	125.1	129.2	114.6
7	124.8	122.1	126.7	128.7	132.4	132.2
8	111.6	111.0	113.0	117.7	118.8	114.6
9	116.4	116.0	122.0	132.3	131.1	128.3
10	120.6	123.1	123.7	125.7	134.7	136.0
11	119.2	124.7	122.8	123.6	137.8	135.4
12	119.4	108.6	120.3	130.7	134.1	129.0

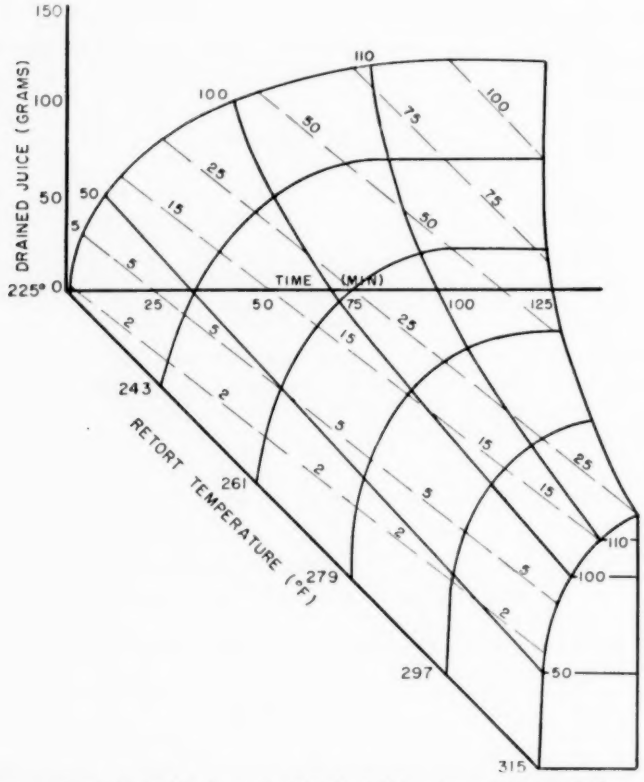

Figure 6. Three-dimensional graph of drained juice, temperature and time.
_____ = lines of equal drained juice.
_____ = lines of equal time.

In Table 9 are shown mean values of processing time, shear force and drained juice for five F_o values for each processing temperature used. The t (processing time) values shown are those which make the process at the given temperature equal to the F_o value designated. Similarly for the shear force and drained juice, the values shown have the same attribute of making the process equivalent to the F_o value shown in the sense that the shear force and drained juice values shown are those which the value derived would indicate to be in existence for the times and temperatures shown. The values for these three attributes at F_o of 4 and 5 for the temperature 225°F. were not included because they would have been the result of extrapolation.

TABLE 9

Mean values of processing time, shear force and drained juice for five F_o values by processing temperatures

Processing temperature	F_o value 1	2	3	4	5
225 °F.					
Time (min.)	74.0	102.0	119.5	.....	.....
Shear force (lb.)	8.8	6.8	5.7	.....	.....
Drained juice (gms.)	108.2	116.8	121.0	.....	.....
243					
Time (min.)	49.0	55.0	59.0	61.5	63.0
Shear force (lb.)	8.8	8.1	7.7	7.4	7.2
Drained juice (gms.)	110.1	112.8	114.6	115.7	116.4
261					
Time (min.)	34.0	37.5	39.5	41.0	42.0
Shear force (lb.)	10.9	10.3	10.0	9.7	9.5
Drained juice (gms.)	111.6	113.9	115.5	116.4	117.0
279					
Time (min.)	23.0	26.0	27.5	28.5	29.5
Shear force (lb.)	11.0	10.3	9.9	9.6	9.4
Drained juice (gms.)	111.7	114.8	116.4	117.2	118.2
297					
Time (min.)	21.0	24.0	5.0	26.0	26.5
Shear force (lb.)	9.5	8.7	8.4	8.1	8.0
Drained juice (gms.)	119.2	122.7	123.9	125.1	123.6
315					
Time (min.)	16.5	17.5	18.0	18.5	18.8
Shear force (lb.)	9.0	8.5	8.2	8.0	7.8
Drained juice (gms.)	122.4	124.0	124.7	125.3	126.0

Of considerable importance in this table is the pattern of time values which are shown for each F_o value. It may be noted that for the lower temperatures, the times required to produce a higher F_o value are considerably longer, as would be expected, than those necessary for lower F_o values. However, as the temperature of processing is raised, the intervals between time values are decreased until at 315°F. the total span between $F_o = 1$ and $F_o = 5$ is only about 2 minutes. This agrees well with theory.

Considering the shear force values, it should be noted that for $F_o = 1$ the values change over processing temperature in such fashion as to pro-

duce a maximum in the middle of the temperature range between about 261 and 279°F. This same pattern is noted throughout the series of F_0 values shown and seems to indicate that the shear force increases with processing temperature up to a maximum at the middle of the temperature range and decreases again at the end. As would be expected, this change is not so marked for high F_0 values as it is for low ones.

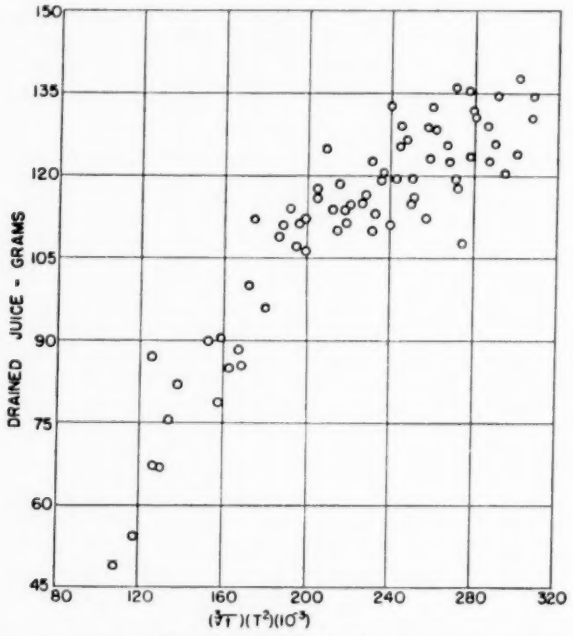

Figure 7. Transformed drained juice relationship for means of three cuts.

Considering the drained juice values, those shown in $F_0 = 1$ indicate that a minimum occurred at 243°F.; the values for 225 and for the higher temperatures being higher than this one. This phenomenon is again noted for the values under F_0 of 2 and 3, but not for F_0 values of 4 and 5, probably because of the missing values for 225°F. In general, with the exception of 225°F. processing, the drained juice values appear to increase with processing temperature.

SUMMARY

Matched sets of round cuts of canner and cutter beef were processed at temperatures ranging from 225-315°F. for 12 times at each processing temperature.

Determination of shear force using the Bratzler-Warner shear for measurement of this attribute after processing, indicates that much variation is encountered in evaluating this attribute with the Bratzler-Warner shear.

It has been found that the rate of change of shear force is a fairly complex function which apparently increases at the beginning of a process in a curvilinear fashion, reaching a maximum and then decreasing roughly linearly throughout the ranges of times studied in this experiment. Further, it was found that the rate of change of shear force in the linear portion of the curve appeared to be exponentially related to temperature.

The equations showing this relationship are

$$\hat{\beta} = 0.04431 \cdot 10^{-0.00894\,(T-200)}$$

in which

$$\beta = \frac{Y - a}{X}$$

and

Y = mean shear force averaged over cuts

X = process time

T = process temperature.

Application of analysis of variance to the shear force data indicated that in only one case out of 12 was there any significant variation in the shear force values contained within a single time set value.

The lack of significance within a time set value shows that the shear force values resulting from use of the design (Figure 1) are roughly equivalent for all of the processing temperatures contained in each time set value. As a result the data were pooled within time set values and used to provide an estimating equation

$$\beta = \frac{-1.5389}{(10 - 8/5\,x)}$$

for the slope of the linear portion of the shear force curve for each processing temperature.

The measurement of drained juice for all the processing times and temperatures used in this experiment has indicated that the values obtained are concordant and may be evaluated in terms of a linear regression of the transformed values. The transform used was designed to include time and temperature together in the results. This treatment yielded the expression

$$Y = 0.33X + 38.02 \text{ or in terms of the transform}$$

$$Y = \frac{t^{1/3}\,T^2}{3030} + 38.02\ .$$

Either one of these equations may be used as a prediction equation to determine in advance approximately the amount of drained juice which should result from a processing time-temperature combination within the limits used in this experiment.

Consideration of changes in shear force and drained juice with sterilizing value of equivalent processes indicated that the firm texture, usually

lacking in canned beef, might best be obtained using processing temperatures between 243-279°F. at F_0 values of 3 or less. The accumulation of drained juice during the process was found to be almost directly proportional to the processing temperature.

LITERATURE CITED

1. BARD, J. C., AND TISCHER, R. G. Objective measurement of changes in beef during heat processing. *Food Technology*, **V** (7), 296-300 (1951).

2. HURWICZ, H., AND TISCHER, R. G. Heat processing of beef. I. A theoretical consideration of the distribution of temperature with time and in space during processing. *Food Research*, **17** (4), 380-392 (1952).

3. HURWICZ, H., AND TISCHER, R. G. Heat processing of beef. II. Development of isothermal and isochronal distributions during heat processing of beef. *Food Research*, **17** (6), 518-542 (1952).

INVESTIGATION OF BARCELONA AND DU CHILLY FILBERT NUTS. III. AMINO ACID AND B VITAMIN CONTENTS OF OIL-FREE FILBERT NUT MEALS[a]

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(Manuscript received March 7, 1953)

In a previous paper *(5)*, two filbert nut globulins were found to be low in lysine and methionine. Using filbert nut globulin as a sole source of protein in an experimental ration weanling rats failed to grow during a 28-day experimental period. However, Cajori *(3)*, using partly defatted ground filbert nuts as the sole source of protein, found a satisfactory growth in young rats. He also found that rats pre-fed on a diet devoid of water-soluble vitamins promptly recovered weight loss when filbert nuts were introduced into the diet. In view of these observations, it was considered desirable to determine the amino acid distribution and B vitamin content of oil-free filbert nut meals, and nutritional adequacy for growing rats on the ration prepared from them.

EXPERIMENTAL

Preparation of oil-free meal. The '46, '47 and '48 crops of Barcelona and Du Chilly filbert nuts *(Corylus Avellana)* were used in this study. The nuts were extracted with petroleum ether to remove the oil and were ground into a fine powder in a Wiley mill. These samples of oil-free filbert nut meals were analyzed for amino acid and B vitamin content. The meals were also used as a source of protein in test rations for rats.

Analytical methods. The Kjeldahl-Gunning method *(1)* was employed to determine the total nitrogen. The microbiological procedure of Henderson and Snell *(7)* with slight modification was used for the assay of amino acids. Microbiological methods employed for the determination of B vitamins were those of Sarett and Cheldelin *(9)* as modified by Fang and Butts *(6)* for thiamine; Krehl, Strong, and Elvehjem *(8)* for nicotinic acid; Skeggs and Wright *(10)* for pantothenic acid; Snell and Strong *(11)* for riboflavin; Tepley and Elvehjem *(12)* for folic acid; and Wright and Skeggs *(13)* for biotin.

Animal feeding experiments. Test rations were made up according to the method of Bubl and Butts *(2)* except that the intact protein at a level of 16.5% was used. Celluflour was omitted in rations containing the filbert nut meal. The composition of these rations is shown in Table 1. The animals were young rats 28 to 30 days of age of the Wistar strain. They were placed in individual wire cages and subjected to paired feeding, using casein as the reference protein.

RESULTS AND CONCLUSION

Results of the microbiological assay for 17 amino acids are summarized in Table 2. The percentage of valine, proline, serine, aspartic acid, and lysine is found to be slightly higher, and cystine, threonine, and tryptophan are found to be lower in the filbert nut meals than in the globulins. The methionine content was found to be the same in both filbert nut

[a]Approved for publication as Technical Paper No. 776 by the Director of the Oregon Agricultural Experiment Station. Contribution of the Department of Agricultural Chemistry.

TABLE 1

Composition of oil-free filbert nut meals and casein rations

Ration No.	1	2	3	4	5	6	7	8	9	10	11	12
	%	%	%	%	%	%	%	%	%	%	%	%
Corn oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cod liver oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Jones salts mix	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Inositol	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Choline-HCl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Liver extract	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cellu flour	2.0	2.0	2.0	2.0	....	....	....	....	....	....	....	....
Sucrose	74.3	74.3	74.3	74.3	57.2	57.4	57.8	57.8	50.4	50.7	51.2	51.2
Casein	16.5	16.3	16.0	16.0	....	....	....	....	....	....	....	....
Barcelona meal[b]	....	....	....	....	35.6	35.2	34.5	34.5	....	....	....	....
Du Chilly meal[c]	....	....	....	....	....	....	....	....	42.4	41.9	41.1	41.1
DL-Tryptophan	....	0.2	....	....	....	0.2	....	....	....	0.2	....	....
L-Cystine	....	....	0.5	....	....	....	0.5	....	....	....	0.5	....
DL-Lysine	....	....	....	0.5	....	....	....	0.5	....	....	....	0.5

[b] Contained 46.3% crude protein.
[c] Contained 38.9% crude protein.

meals and globulins. The filbert nut meals, when compared to casein, were high in arginine and serine, and low in lysine, proline, tyrosine, and methionine. However, the amino acids were rather well balanced for a plant protein.

The contents of B vitamins are summarized in Table 3. The B vitamin contents of a few other foodstuffs obtained from literature *(4)* are also included in this table for comparison. It is obvious that the vitamin contents of filbert nut meals vary slightly in different crops. In general, they are very rich in all the B vitamins determined.

TABLE 2

Amino acid composition of filbert nut meals

Amino acid[d]	Assay organism[e]	Barcelona[f] variety	Du Chilly[f] variety
		%	%
Arginine	S	15.57	14.71
Aspartic acid	M	7.00	7.35
Cystine	M	0.43	0.43
Glutamic acid	A	21.00	20.10
Glycine	M	9.18	9.80
Histidine	S	1.77	1.80
Isoleucine	A	5.64	6.94
Leucine	A	6.16	6.25
Lysine	M	2.82	3.14
Methionine	S	0.71	0.90
Phenylalanine	A	3.55	3.46
Proline	M	5.73	5.50
Serine	M	9.18	10.14
Threonine	S	2.70	2.64
Tryptophan	A	1.08	1.13
Tyrosine	S	3.70	3.76
Valine	S	6.15	6.65

[d] The amino acids used as standards were dried in vacuo at 45° C. and kept in a desiccator containing anhydrous calcium chloride. The DL forms of tryptophan, threonine, phenylalanine, serine, methionine, leucine, isoleucine, and valine were employed; the natural isomers of the other acids were used.

[e] S = *Streptococcus faecalis* R. A = *Lactobacillus arabinosus* 17-5. M = *Leuconostoc mesenteroides* P-60.

[f] Results were calculated to 16.0 g. of nitrogen.

The comparative results of growth response of young rats on rations containing casein or filbert nut meals as the source of protein with or without supplements are summarized in Table 4. It is obvious that the protein from both filbert nut meals is inferior in quality to that of casein. Du Chilly filbert nut protein promoted growth somewhat better than that of the Barcelona variety. In both cases, supplementing with 0.2% DL-tryptophan or 0.5% L-cystine showed a slight improvement. When supplemented with 0.5% DL-lysine, a distinct improvement resulted and was almost equal to the casein ration which was supplemented with the like amount of DL-lysine, in promoting growth. Nevertheless, the filbert meal rations even without supplementation still appeared to promote better growth than the stock ration. This stock ration has a protein level of approximately 19.5%. It is undoubtedly true that the filbert nut meal protein is well balanced for a plant protein as indicated in the results from amino acid analyses.

TABLE 3

B vitamin contents in filbert nut meals (γ/g. of dry sample)

	Thiamine	Nicotinic acid	Riboflavin	Pantothenic acid	Folic acid	Biotin
Barcelona variety						
1946 crop	17.1(4)[a]	27.7(4)	5.14(4)	36.8(4)	0.66(4)	1.14(4)
1947 crop	15.6(1)	30.9(1)	6.15(1)	39.5(1)	0.85(1)	1.14(1)
1948 crop	13.9(3)	21.8(3)	5.75(3)	28.8(3)	0.74(3)	1.01(3)
Du Chilly variety						
1946 crop	13.5(4)	28.2(4)	6.13(4)	32.1(4)	0.81(4)	1.21(4)
1947 crop	11.1(1)	26.4(1)	6.31(1)	39.6(1)	1.48(1)	1.16(1)
1948 crop	18.1(3)	25.1(3)	6.33(3)	33.0(3)	1.05(3)	1.32(3)
Brewers' yeast[b]	8.5	126.0	15.2	42.5	1.05	0.071
Whole wheat[b]	5.6	46.0	1.8	13.0	2.0	0.058
Lima bean seed[b]	5.8	11.0	1.4	9.0	3.6	0.11
Whole milk[b]	3.6	5.5	7.8	24.0	<0.4	0.41
Beef (round)[b]	2.1	160.0	7.5	17.0	3.4	0.077
Roast peanuts[b]	14.0	88.0	1.1	25.0	2.9	0.35

[a] Numbers in parentheses denote the number of samples analyzed.
[b] Cheldelin, V. H., and R. J. Williams, *The B Vitamin Content of Foods*, The University of Texas *Publication No. 4237*, 105 (1942).

TABLE 4

Comparative results of growth response of young rats on rations containing casein or filbert nut meal as the source of protein with or without supplements

Rations compared	Number of pairs	Average food intake	Food intake/weight gained	
			Casein diet	Filbert meal diet
		gm.		
1	3	261	2.91	....
1 to 5	2	339	3.01	4.98
1 to 9	2	303	3.01	4.26
2 to 6	2	247	3.19	4.34
2 to 10	2	288	3.19	3.79
3 to 7	2	233	2.74	4.31
3 to 11	2	260	2.74	3.72
4 to 8	2	217	2.77	3.08
4 to 12	2	253	2.77	2.97

SUMMARY

Seventeen amino acids and B vitamin contents of Barcelona and Du Chilly filbert nut meals were determined by microbiological assay methods. The amino acids of filbert nut protein are rather well balanced, although somewhat low in lysine, methionine, proline, and tyrosine as compared to casein. When supplemented with 0.5% DL-lysine, the filbert nut meal rations were found almost equal nutritionally to a casein ration in promoting growth of young rats. The filbert nut meals were very rich in all the B vitamins and especially so in biotin.

LITERATURE CITED

1. Association of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*, 6th edition, 1945, Washington, D. C.

2. Bubl, E. C., and Butts, J. S. The utilization of D-tyrosine for growth in rats. *J. Biol. Chem.*, **174**, 637 (1948).
3. Cajori, F. A. Some nutritive properties of nuts; their proteins and content of water soluble vitamins. *J. Biol. Chem.*, **43**, 583 (1920).
4. Cheldelin, V. H., and Williams, R. J. *The B Vitamin Content of Foods.* University of Texas Publication No. 4237, 105 (1942).
5. Fang, S. C., and Butts, J. S. Investigation of Barcelona and Du Chilly Filbert Nuts. II. Isolation, nutritional evaluation and amino acid distribution of filbert globulins. *J. Nutr.*, **40**, 329 (1950).
6. Fang, S. C., and Butts, J. S. Microbiological assay method for thiamine using *Lactobacillus fermenti* 36 as test organism. *Proc. Soc. Exptl. Biol. Med.*, **78**, 463 (1951).
7. Henderson, L. M., and Snell, E. E. A uniform medium for determination of amino acids with various microorganisms. *J. Biol. Chem.*, **172**, 15 (1948).
8. Krehl, W. A., Strong, F. M., and Elvehjem, C. A. Determination of nicotinic acid. Modifications in the microbiological methods. *Ind. Eng. Chem., Anal. Ed.*, **15**, 471 (1943).
9. Sarett, H. P., and Cheldelin, V. H. The use of *Lactobacillus fermenti* 36 for thiamine assay. *J. Biol. Chem.*, **155**, 153 (1944).
10. Skeggs, H. R., and Wright, L. D. The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. *J. Biol. Chem.*, **156**, 21 (1944).
11. Snell, E. E., and Strong, F. M. A microbiological assay for riboflavin. *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939).
12. Tepley, L. J., and Elvehjem, C. A. The titrametric determination of *Lactobacillus casei* factor and folic acid. *J. Biol. Chem.*, **157**, 303 (1945).
13. Wright, L. D., and Skeggs, H. R. Determination of biotin with *Lactobacillus arabinosus*. *Proc. Soc. Exptl. Biol. Med.*, **56**, 95 (1944).

OXIDATIVE FAT RANCIDITY IN FOOD PRODUCTS. I. LINOLEATE OXIDATION CATALYZED BY HEMIN, HEMOGLOBIN, AND CYTOCHROME C[a]

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(Manuscript received April 17, 1953)

The important role that hematin compounds play in the oxidation of unsaturated fats in animal products has been demonstrated. The catalysis of unsaturated fat oxidation in pork products has been ascribed to the presence of hematin compounds *(7, 8, 12)*. In fish, poultry, and other animal tissues hematin appears to be the predominant catalyst for oxidation of linoleic acid *(9)*.

The ubiquitous distribution of hematin compounds raises the question of their importance in the oxidative fat rancidity occurring in many other products. Hemoglobin and myoglobin occur in relatively high concentration in animal tissues. Cytochrome c which serves the important function of transferring electrons for biological oxidations has a universal distribution in animals, plants, and microorganisms. Thus it might be asked, is the oxidation of unsaturated fats by certain spoilage microorganisms due to the high content of the cytochromes? Does hematin act as a catalyst in oxidative fat rancidity of nut meats?

There is little fundamental information on hematin catalysis of unsaturated fat oxidation in which variables of importance to food preservation are considered. It would be interesting to know the effect of temperature upon hematin catalysis because of the widespread use of refrigerated and frozen preservation. Chang and Watts *(2)* found antioxidants to be effective inhibitors of the hemoglobin-catalyzed oxidation of lard. The use of antioxidants appears to be the best method available for the prevention of oxidative fat rancidity which might be catalyzed by hematin compounds. Consequently, this information should be extended. The bleaching of meat pigments may be due to co-oxidation of hemoglobin and myoglobin in the presence of oxidizing unsaturated fats *(13)*. Haurowitz, Yenson, and Schwerin *(3)* showed the destruction of hemin and hemoglobin at physiological temperature. The rate of destruction of hematin catalysts and the effect of their co-oxidation on unsaturated fatty acid oxidation is not known.

It was the purpose of this investigation to determine the effect of temperature, antioxidants, and co-oxidation of the heme group on linoleate oxidation in the presence of various hematin catalysts, including hemin, cytochrome c, and hemoglobin. A very low activation energy was found for cytochrome c and hemoglobin catalysis. Antioxidants were effective in preventing initiation of linoleate oxidation. Hemin was rapidly co-oxidized in the linoleate system at 0°C.

[a] Presented before the Thirteenth Annual Meeting of the IFT, Boston, Massachusetts, on June 23, 1953.

EXPERIMENTAL

Hematin catalysts. Hemoglobin solution was prepared from fresh cattle blood by shaking with glass beads to defibrinate, washing the red blood cells with saline 4 times, and laking with distilled water. Hemoglobin and cytochrome c (Sigma Chemical Co.) were standardized against crystalline hemin (Armour and Co.) by conversion to pyridine hemochrome and determination of the spectral absorption at 560 mμ.

Linoleate and antioxidants. Highly purified linoleic acid (Hormel Foundation) was neutralized with ammonium hydroxide to form a homogeneous 0.1 M linoleate solution and stored under pure nitrogen at −20°C. Colloidal linoleate was prepared by suspending ammonium linoleate in 0.1 M phosphate buffer at pH 7.0. Ascorbic acid and α tocopherol were reagent grade. Propyl gallate and nordihydroguaiaretic acid (N.D.G.A.)[b] were commercial preparations.

Oxygen absorption measurements. The majority of these studies were carried out at 0°C. using a refrigerated Warburg respirometer. The main compartment of the reaction flask contained colloidal linoleate at a concentration which resulted in 0.02 M linoleate in the final reaction mixture of 4 ml. One ml. of aqueous hematin catalyst was placed in one side arm and mixed with the linoleate after gassing with pure O_2 and temperature equilibration. Antioxidants dissolved in 0.1 ml. ethyl alcohol or water were placed in the second side arm.

Oxidation of pork adipose tissue. Triplicate samples of 3.2 g. of minced uncured side pork adipose in each respirometer flask were allowed to oxidize in an atmosphere of oxygen at 0°C. The concentration of hematin compounds in the pork adipose tissue was determined by extraction with water and conversion to the pyridine hemochrome.

Hemin co-oxidation. The respirometer flasks were removed from the Warburg apparatus at a predetermined amount of oxygen absorption. The reaction was immediately stopped by addition of 1 ml. of 10% hydroxylamine hydrochloride to the flask contents. The resulting mixture was quantitatively transferred to a graduated test tube with the aid of a distilled water rinse. One ml. of 0.1% o-phenanthroline and 2 ml. of 1% acetic acid in a 2:3 volume ratio of butanol-ethyl ether were added to the aqueous phase. The volume of the aqueous phase was adjusted to 10 ml., the organic phase was adjusted to 5 ml., and the phases were separated after equilibration. The o-phenanthroline-ferrous complex in the aqueous phase was determined using a 4-cm. cell in a Klett colorimeter with a 400-465 mμ filter. Acid hematin in the butanol-ethyl ether was determined using standard Klett tubes and a 400-465 mμ filter. These methods were standardized using crystalline hemin and reagent iron wire.

RESULTS AND DISCUSSION

The oxidation of linoleate at 0°C. catalyzed by hematin compounds. The temperature of 0°C. was selected in these studies to simulate more closely the temperature conditions of refrigerated and frozen preservations of foods. This low temperature also decreased, as much as possible, the autocatalytic oxidation of linoleate.

The effect of increasing concentrations of hemin, hemoglobin, and cytochrome c on the total oxidation is shown in Figures 1, 2, and 3. The concentrations of the hematin catalysts are in general less than their concentrations in biological systems. For comparison, the hemoglobin content of blood is 2×10^{-3} M, and the cytochrome c content of muscle ranges from 8×10^{-7} M for rabbit muscle to 4×10^{-5} M for pigeon breast *(6)*. The total concentration of hematin compounds including hemoglobin, myoglobin, and cytochromes in lean pork muscle was approximately 3×10^{-4} M *(8)*.

The rates of linoleate oxidation in the presence of hemin, hemoglobin, and cytochrome c as shown in Figures 1, 2, and 3 indicated that these hematin compounds are relatively powerful catalysts. With the exception

[b] N.D.G.A. was generously supplied by Wm. J. Stange Co.

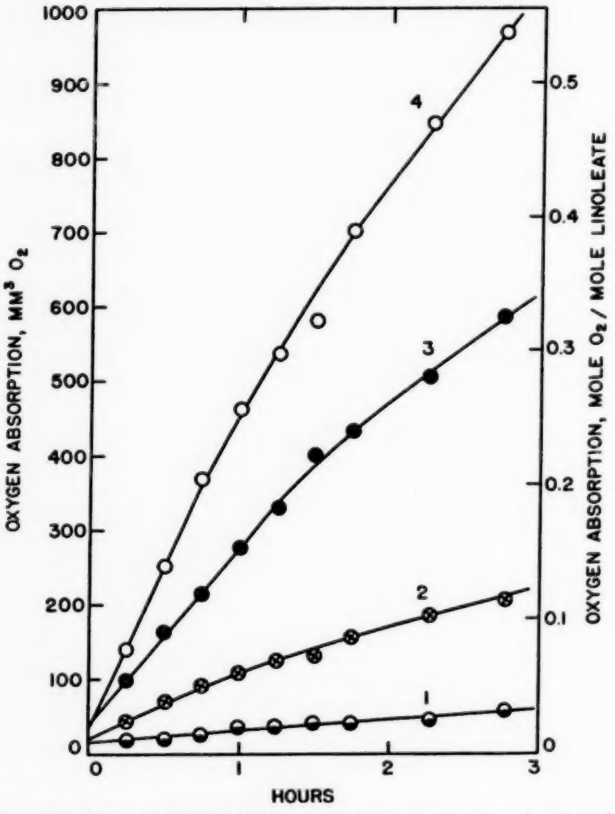

Figure 1. The effect of initial hemin concentration on the rate of colloidal linoleate oxidation at 0°C.

1. 0.38 × 10^{-6} M Hemin
2. 3.8 × 10^{-6} M Hemin
3. 29 × 10^{-6} M Hemin
4. 77 × 10^{-6} M Hemin

of the enzyme lipoxidase, the hematin compounds appear to be the most active naturally occurring catalysts for unsaturated fat oxidation at low temperature. The rate of hematin-catalyzed linoleate oxidation, while much less than that of the enzyme lipoxidase, is large compared to autocatalysis. The rates for cytochrome c and hemoglobin catalysis at 0°C. are 38 moles linoleate oxidized/(mole cytochrome c)$^{1/2}$/mole linoleate/hour and 152 moles linoleate oxidized/(mole hemoglobin)$^{1/2}$/mole linoleate/hour,[c] respectively. From the turn-over number of lipoxidase under optimum conditions at 20°C. *(4)* and the activation energy *(10)* the rate of linoleate oxidation at 0°C. was calculated to be 1 × 10^4 moles linoleate oxidized/mole lipoxi-

[c] A mole of hemoglobin of mol. wt. 68,000 containing 4 heme groups is used throughout.

dase/hour. Studies of autocatalytic linoleate oxidation have not been made at 0°C., but data on autoxidation at elevated temperatures indicate a much lower oxidation rate than hematin catalysis. For autoxidation at 35°C. the rate was about 2.8×10^{-2} mole ethyl linoleate oxidized/mole linoleate/mole linoleate peroxide/hour *(1)*. Utilizing the activation energy of 17.2 kilocalories/mole an extrapolation to 0°C. gives 7.6×10^{-4} mole ethyl linoleate oxidized/mole linoleate/mole linoleate peroxide/hour. It should be emphasized that this comparison of hematin catalysis with enzymic catalysis and autocatalysis was made only to illustrate the relative rates of these catalytic oxidations. The mode of action of these 3 catalysts is considerably different.

The data of Figures 1, 2, and 3 also show the effect of hematin compound destruction that occurs during the course of linoleate oxidation. As

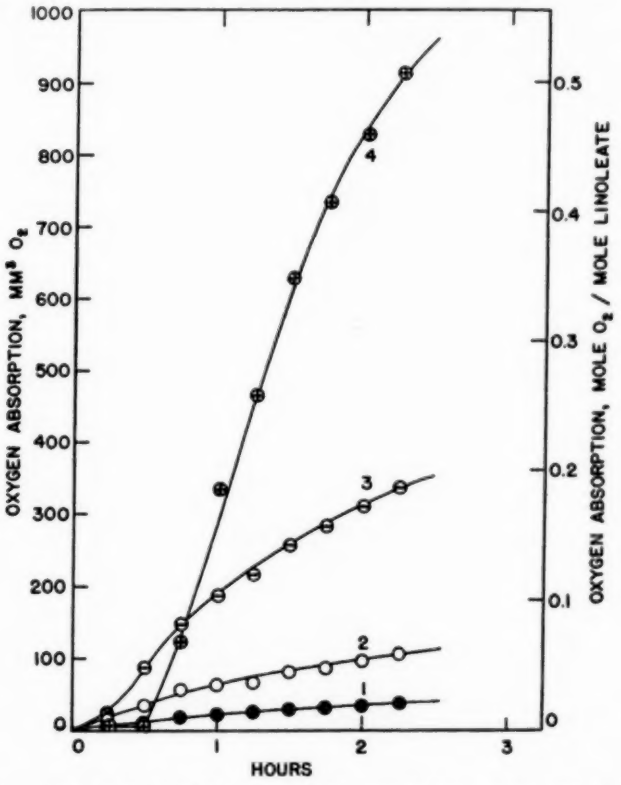

Figure 2. The effect of initial hemoglobin concentration on the rate of colloidal linoleate oxidation at 0°C.

1. 0.03×10^{-6} M Hemoglobin
2. 0.4×10^{-6} M Hemoglobin
3. 2.5×10^{-6} M Hemoglobin
4. 10×10^{-6} M Hemoglobin

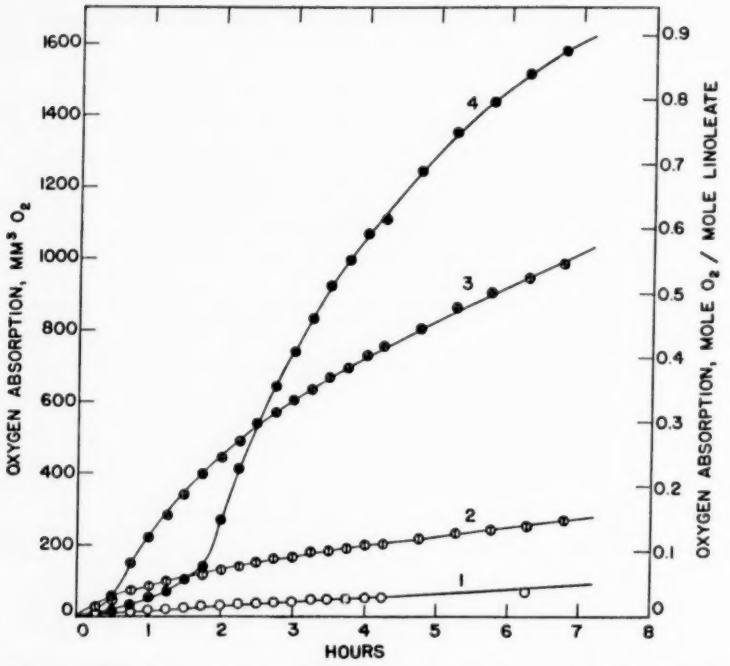

Figure 3. The effect of initial cytochrome c concentration on the rate of colloidal linoleate oxidation at 0°C.

1. 0.25×10^{-6} M Cytochrome c
2. 3.0×10^{-6} M Cytochrome c
3. 30×10^{-6} M Cytochrome c
4. 80×10^{-6} M Cytochrome c

the oxidations progressed the rate of oxygen absorption decreased. In general, this decrease in reaction rate could be attributed to the destruction of the hematin catalysts. The important topic of the destruction of hematin compounds in an oxidizing linoleate system will be considered in more detail later. Only in the case of hemoglobin at concentrations of about 2×10^{-5} M and above did the co-oxidation of the hematin catalyst appear to cause initial oxygen absorption greater than that which could be accounted for by linoleate oxidation. In all other cases the co-oxidation of a small amount of hematin catalyst did not significantly affect the determination of the rate of linoleate oxidation by measurement of oxygen absorption.

The inhibition periods at high concentrations of hemoglobin and cytochrome c and the relative catalytic activities of hemin, hemoglobin, and cytochrome c are discussed elsewhere *(11)*.

Hematin catalysis as the mechanism of pork fat oxidation. Knowledge of the rates of linoleate oxidation in these simple systems containing hematin compounds allows a test of the hypothesis that the hematin compounds

are the catalysts responsible for the rapid oxidation of the unsaturated fats during refrigerated or frozen storage of animal tissues. The case of pork fat oxidation may be considered as a typical example. The problem of oxidative fat rancidity of pork has been extensively investigated. Three possible mechanisms may account for oxidative fat rancidity in pork tissue: autocatalysis, hematin catalysis, and enzymic catalysis. Studies with extracts of pork tissue *(8, 12)* have clearly indicated that hematin catalysis of unsaturated fat oxidation is the most probable cause of oxidative rancidity. The possibility of enzymic activity appears slight since no enzyme capable of causing direct oxidation of homogenous linoleate could be demonstrated *(8)*. Because the hematin content of tissue extracts can be correlated fairly well with their linoleate oxidation catalytic activity in simple systems, it would be interesting to extend these observations to the more complex natural systems.

In this research, the oxidation rate and hematin content of minced uncured side pork adipose were determined. At 270 hours the oxygen absorption was equivalent to the oxidation of approximately 5% of the dienoic fatty acids. The course of the reaction shown in Figure 4 might

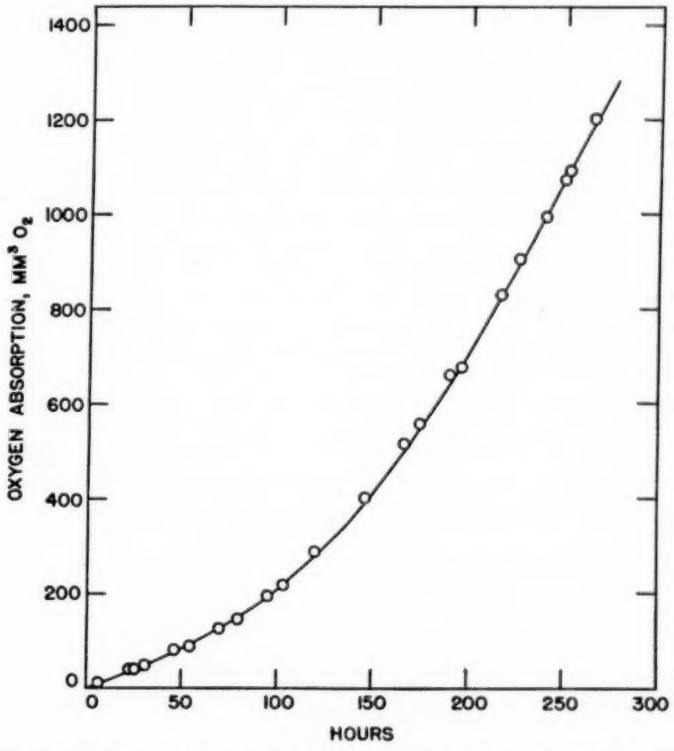

Figure 4. Oxidation at 0°C. of pork adipose tissue containing 1×10^{-6} M hematin.

suggest an autocatalytic reaction except for a comparatively large initial reaction rate. If it were assumed that the mechanism for oxidation of the pork fat were autocatalytic, the rate of oxidation could be compared to the rate of autocatalytic oxidation of pure ethyl linoleate. In calculating the rate of pork adipose oxidation it was assumed that the more reactive dienoic fatty acid glycerides would be oxidized first and that the more saturated fatty acid glycerides would act merely as diluents. The experimental value of 8.2 moles dienoic fatty acid oxidized/mole O_2 absorbed/mole dienoic fatty acid/hour for pork adipose oxidation is 1×10^4 times higher than ethyl linoleate autocatalysis in a simple system. Likewise, when the rate of pork adipose oxidation was compared with the autocatalysis of ethyl linolenate or methyl arachidonate *(5)* the rate of pork adipose was very much larger. It is therefore apparent that the rate of oxidation of pork adipose is many orders of magnitude greater than that of autocatalytic oxidation of the unsaturated fatty acids known to be present in pork fat. In contrast, the assumption that hematin-catalyzed oxidation is the predominant reaction shows full agreement with experimental observations. The equivalent hemin concentration of 1×10^{-5} M found to be present in the pork adipose is more than enough to account for the initial reaction rate of 9×10^{-5} moles dienoic fatty acid oxidized/mole dienoic fatty acid/hour. Hematin catalyzed oxidation of unsaturated fats could produce an exponential type curve (Figure 4) if there were a time lag prior to the attainment of the maximum oxidation rate. The time required for build-up of a sufficient concentration of peroxides for maximum rate depends upon the reactivity of the unsaturated fatty acid *(11)*. In the solid pork adipose comparatively low diffusion rates would be expected to control the over-all oxidation rate and the time required for build-up of peroxide concentration.

Activation energy for hemoglobin and cytochrome c catalyzed oxidation of linoleate. The data from a large number of determinations of the initial rate of linoleate oxidation in the presence of various concentrations of hemoglobin and cytochrome c have been used to evaluate the activation energies as shown in Figure 5. The activation energy of cytochrome c catalysis was based upon 33 determinations of the rate of linoleate oxidation with cytochrome c varying from 2×10^{-7} M to 8×10^{-5} M. The activation energy for hemoglobin catalysis was based upon 15 determinations with a hemoglobin concentration range of 3×10^{-8} M to 5×10^{-6} M. The activation energy of 3.3 kilocalories/mole for hemoglobin and cytochrome c catalysis is very low. For comparison, autocatalytic linoleate oxidation in the same aqueous colloidal system has an activation energy of 15.2 kilocalories/mole and ethyl linoleate autoxidation in a single-phase system has an activation energy of 17.2 kilocalories/mole *(1)*.

The importance of this low activation energy of hematin catalysis may be better demonstrated by comparing the reduction of reaction rates. If autocatalysis and hematin catalysis proceeding at 37°C. were reduced to a temperature of 0°C. autocatalysis would be reduced 98% while hematin catalysis would be reduced only 55%. The very low activation energy of 3.3 kilocalories/mole for hematin catalyzed linoleate oxidation suggests that a lowering of storage temperature will cause relatively little reduction of unsaturated fat oxidation in food products where hematin catalysis

predominates. This information may explain the difficulties encountered in unsaturated fat oxidation in pork, fish, and poultry in frozen storage. The frozen preservation of these products at conventional frozen-food temperatures must be augmented with exclusion of oxygen and sometimes the addition of antioxidants in order to obtain a desirable storage life.

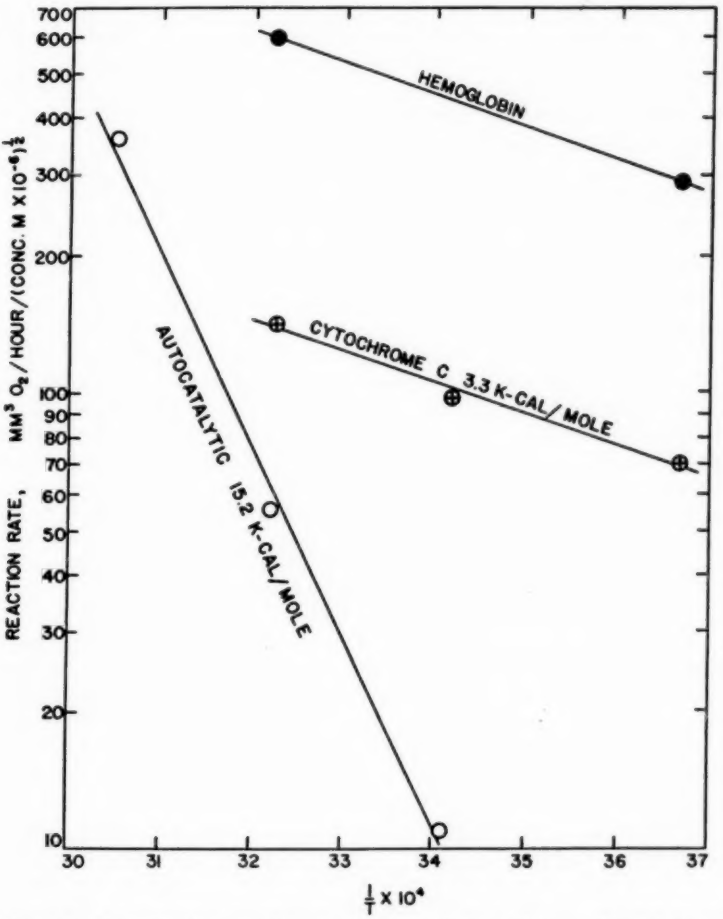

Figure 5. The activation energies of colloidal linoleate oxidation catalyzed by cytochrome c and hemoglobin compared to autocatalytic linoleate oxidation.

If it were impossible to give added protection by packaging, wrapping, or glazing to exclude oxygen or to effectively add antioxidants, a frozen food product undergoing hematin catalyzed unsaturated fat oxidation could have its storage life extended only by a relatively large reduction of storage temperature.

Because of the complex physical and biochemical nature of foods the knowledge of the activation energy of a predominant spoilage mechanism does not allow a prediction of storage life in quantitative terms. In a frozen food undergoing hematin catalyzed unsaturated fat oxidation the rate of diffusion of the reactants to and the counterdiffusion of products away from the catalytic hematin surface will determine the overall rate of fat oxidation. The proportion of frozen phase, and hence, the diffusion barrier for unsaturated fat and oxygen will depend upon the storage temperature. It is well known that foods stored at temperatures of 0°C. to −20°C. may have considerable amounts of eutectic mixtures of inorganic salts, organic compounds, and water which remain in the liquid phase.

The inhibition of hematin catalysis by antioxidants. Because of the biological importance of the vitamins, ascorbic acid and α-tocopherol, it was of interest to determine the effect of their presence in the systems of hematin catalyzed linoleate oxidation. Besides the biological antioxidant, α-tocopherol, the 2 widely used commercial antioxidants, nordihydroguaiaretic acid (N.D.G.A.) and propyl gallate were also used.

The hemin-catalyzed linoleate oxidation was completely inhibited by the presence of 2×10^{-3} M α-tocopherol, N.D.G.A., or propyl gallate when these antioxidants were initially present (Figure 6). However, if the catalyzed oxidation were allowed to proceed for one hour before the addition of antioxidant, the oxidation could not be stopped by these relatively large amounts of the antioxidants. Apparently the hemin-catalyzed linoleate oxidation was readily inhibited by the initial interruption of chain reactions. Initially, there were few reaction chains and the antioxidants due to their property of easily donating electrons and forming stable and nonpropagating semiquinones effectively broke these reaction chains. Since hemin catalysis is dependent upon the formation of linoleate peroxides *(11)* the initial presence of antioxidants produces very effective inhibition. Also little antioxidant was oxidized in the process, as shown by the absence of significant oxygen absorption. In the actively oxidizing hemin-linoleate system, which was allowed to proceed for one hour, there was a comparatively large number of propagating reaction chains. The hematin catalysis of linoleate is characterized by a chain-reaction propagation of relatively short chain lengths *(11)*. The addition of the 3 antioxidants to this actively oxidizing system was thus ineffective in stopping oxidation. Instead the antioxidants themselves were oxidized at an appreciable rate.

Addition of ascorbic acid to the hemin-catalyzed linoleate oxidation system after one hour caused an increase in oxygen absorption. The effect of ascorbic acid was best shown in studies of cytochrome c catalyzed linoleate oxidation (Figure 7). When ascorbic acid was present initially, the rate of total oxidation was decreased, showing that its reducing action has some effect on the catalyzed linoleate oxidation. In actively oxidizing systems the addition of ascorbic acid causes a large increase in total oxidation as shown by curves 4 and 5 in Figure 7. The fact that ascorbic acid is catalytically oxidized by cytochrome c *(6)* cannot account for these large increases in total oxidation. Rather it appears that the rapidly oxidizing linoleate causes co-oxidation of a considerable portion of the ascorbic acid.

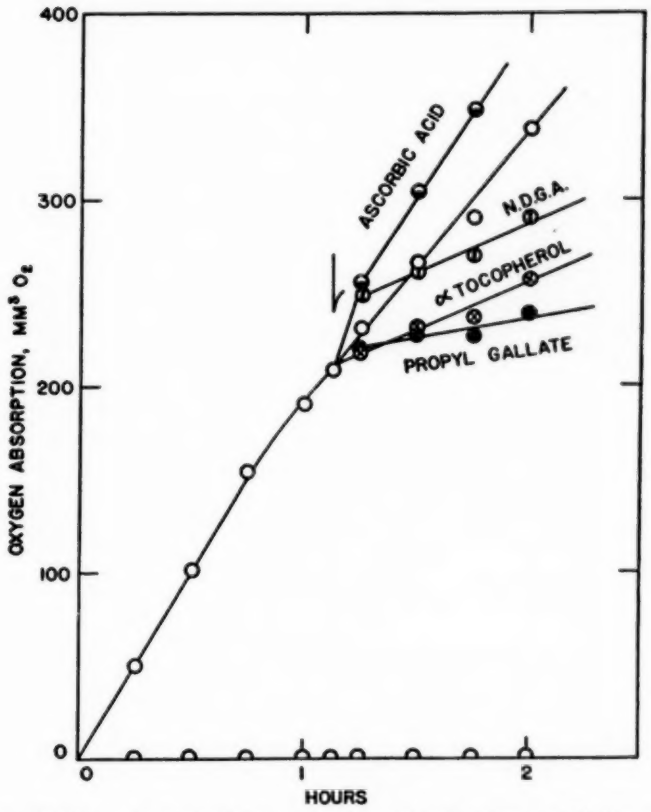

Figure 6. Inhibition by antioxidants of hemin catalyzed linoleate oxidation at 0°C.
Initial hemin concentration was 20×10^{-6} M.
Concentration of all antioxidants was 2×10^{-5} M.

In the cytochrome c catalysis the addition of the antioxidants α-tocopherol, N.D.G.A., and propyl gallate after one hour gave complete inhibition of the linoleate oxidation. In this case, the concentration of cytochrome c was 20 times lower than the concentration of hemin, and linoleate oxdiation rate was much less.

Co-oxidation of the hemin catalyst during linoleate oxidation. During the course of an extended linoleate oxidation the destruction of hemin was measured. Figure 8 shows the decrease of linoleate oxidation rate with increasing time and level of oxidation. Determination of hemin as acid hematin indicated a rapid loss of the hemin molecule.

Simultaneous determinations of the iron ions released showed in Figure 9 that the decrease in hemin could not be accounted for by the iron set free. Apparently the iron was bound to the protoporphyrin during part of its oxidative degradation. It will be noted that about 20% of the hemin

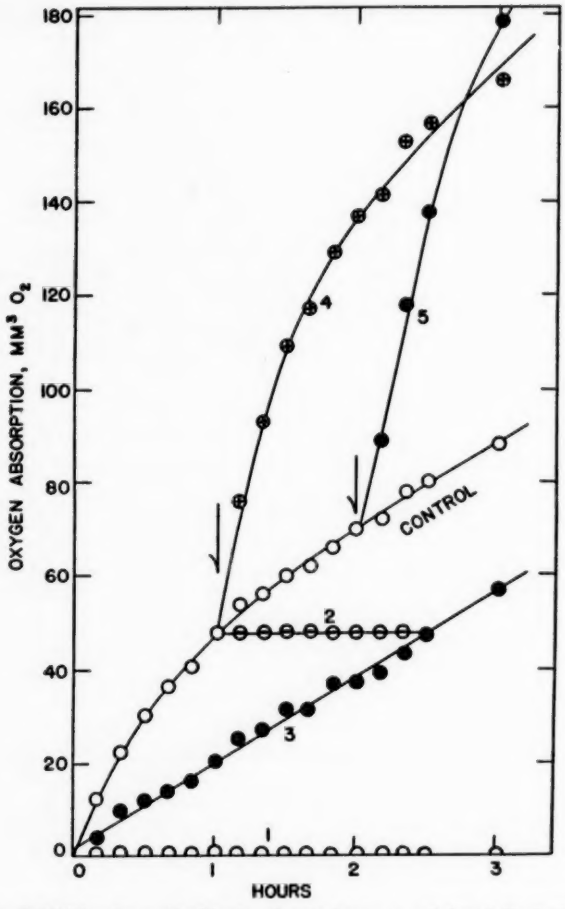

Figure 7. Inhibition by antioxidants of cytochrome c catalyzed linoleate oxidation at 0°C.

Initial Cytochrome c concentration was 1×10^{-6} M.

1. Addition of 2×10^{-3} M α-Tocopherol, propyl gallate, or N. D. G. A. at 0 time.
2. Addition of 2×10^{-3} M α-Tocopherol, propyl gallate, or N. D. G. A. at 1 hour.
3. Addition of 2×10^{-3} M Ascorbic acid at 0 time.
4. Addition of 2×10^{-3} M Ascorbic acid at 1 hour.
5. Addition of 2×10^{-3} M Ascorbic acid at 2 hours.

was lost and 10% of iron released during the initial stages of the linoleate oxidation. This co-oxidation of hemin appears to be responsible for the anomolous rapid oxygen absorption noted in the measurements of linoleate oxidation rates (Figure 1). This initial co-oxidation of hemin was characterized by the release of a greater percent of the equivalent iron than occurred in hemin co-oxidation at higher levels of linoleate oxidation. It has been previously shown that the rate of linoleate oxidation is a function

of the square root of the concentration of hematin catalyst *(8, 11)*. This same relationship holds approximately for the case of a linoleate oxidation in which the hemin was destroyed during the course of the oxidation. Figure 10 shows the correlation between rate of oxidation and concentration of hemin remaining.

The linear relationship between the rate of linoleate oxidation and the square root of hemin remaining was evidence that hemin remained the predominant catalyst during the course of this oxidation. It has been previously shown by Haurowitz, Yenson, and Schwerin *(3)* that the iron released has a negligible catalytic activity compared to hemin. At levels of oxygen absorption greater than 0.8 mole O_2/mole linoleate the decrease of linoleate had an adverse effect on the rate of oxidation. For this reason no significance can be attached to the intersection on the abscissa (Figure 10) at a positive hemin concentration.

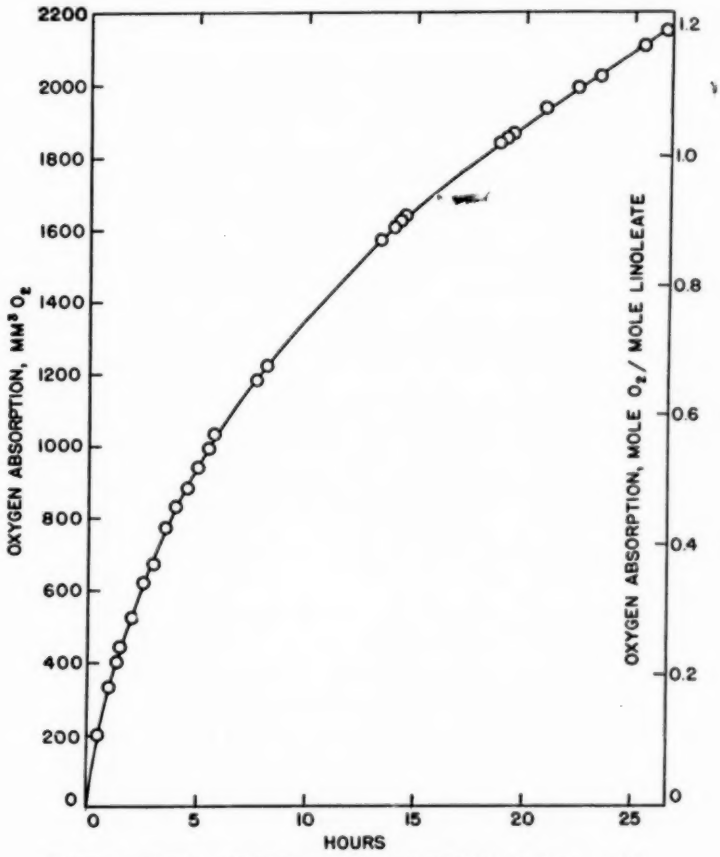

Figure 8. Hemin catalyzed oxidation of colloidal linoleate at °C. Initial hemin concentration was 77 × 10^{-6} M.

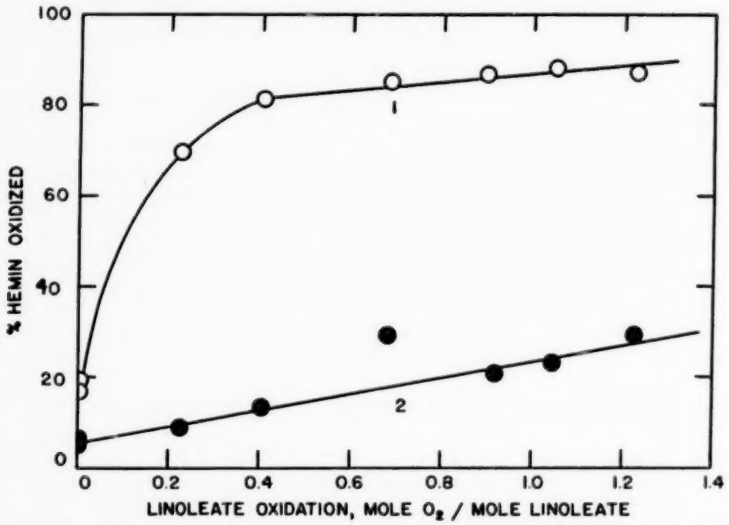

Figure 9. Co-oxidation of hemin during catalysis of colloidal linoleate oxidation.

1. Disappearance of hemin measured by decrease in acid hematin.
2. Disappearance of hemin measured by release of inorganic iron.

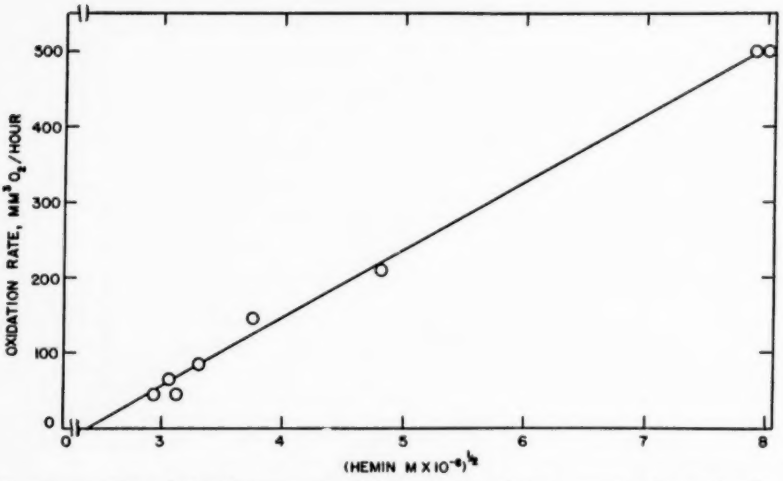

Figure 10. The relation between the oxidation rate and the concentration of hemin.

SUMMARY

Hemin, hemoglobin, and cytochrome c are powerful catalysts for the oxidation of colloidal linoleate at 0°C. The rate of oxidation of pork adipose tissue can be accounted for on the basis of its content of hematin

compounds but not as a simple autoxidation process. Linoleate oxidation catalyzed by hematin compounds has a relatively low activation energy of 3.3 kilocalories/mole. Polyphenolic antioxidants inhibit the initiation of hemin and cytochrome c catalyzed linoleate oxidation. Hemin was co-oxidized and iron released during linoleate oxidation.

Acknowledgment

The author wishes to express his thanks to Merle R. Emerson and A.M.S.A. Siddiqi for their laboratory assistance.

LITERATURE CITED

1. Bolland, J. L., and Gee, G. Kinetic studies in the chemistry of rubber and related materials. II. The kinetics of oxidation of unconjugated olefins. *Trans. Faraday Soc.*, **42**, 236 (1946).
2. Chang, I., and Watts, B. M. Antioxidants in the hemoglobin catalyzed oxidation of unsaturated fats. *Food Technol.*, **3**, 332 (1949).
3. Haurowitz, F., Schwerin, P., and Yenson, M. M. Destruction of hemin and hemoglobin by the action of unsaturated fatty acids and oxygen. *J. Biol. Chem.*, **140**, 353 (1941).
4. Holman, R. T., and Bergstrom, S. Lipoxidase or unsaturated-fat oxidase. *The Enzymes*, Vol. II, Part 1, 559 (1951), Academic Press Inc., New York.
5. Holman, R. T., and Elmer, O. C. The rates of oxidation of unsaturated acids and esters. *J. Am. Oil Chem. Soc.*, **24**, 127 (1947).
6. Lemberg, R., and Legge, J. W. *Hematin Compounds and Bile Pigments.* 1949, Interscience Publishers Inc., New York.
7. Reiser, R. Peroxidizing and carotene bleaching substances in bacon adipose tissue. *J. Am. Oil Chem. Soc.*, **26**, 116 (1949).
8. Tappel, A. L. Linoleate oxidation catalyzed by hog muscle and adipose tissue extracts. *Food Research*, **17**, 550 (1952).
9. Tappel, A. L. Linoleate oxidation catalysts occurring in animal tissues. *Food Research*, **18**, 104 (1953).
10. Tappel, A. L., Lundberg, W. O., and Boyer, P. D. Effect of temperature and antioxidants upon the lipoxidase-catalyzed oxidation of sodium linoleate. *Arch. Biochem.*, **42**, 293 (1953).
11. Tappel, A. L. The mechanism of the unsaturated fatty acid oxidation catalyzed by hematin compounds. *Arch. Biochem.*, **44**, 378 (1953).
12. Watts, B. M., and Peng, D. Lipoxidase activity of hog hemoglobin and muscle extract. *J. Biol. Chem.*, **170**, 441 (1947).
13. Watts, B. M., and Peng, D. Rancidity development in raw versus precooked frozen pork sausage. *J. Home Ec.*, **39**, 88 (1947).

A NEW B VITAMIN STUDY OF WALNUTS

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(Manuscript received April 23, 1953)

A report on the thiamine, riboflavin, and niacin content of 3 varieties of California-grown walnuts of 4 successive crops was published in 1949 *(8)*. Further studies in this laboratory have now been completed which have included analyses for pantothenic acid, folic acid, and vitamin B_6 as well as repetitions of the analyses for the three B vitamins previously studied.

The new samples of *Juglans regia* were grown in the same districts and were shipped to the laboratory in the shells in the same manner previously described.[a] The 3 varieties, Payne, Placentia, and Franquette, were the same used in the first study and samples of the 1950 and 1951 crops were examined.

METHODS

The nuts were shelled, ground thoroughly twice, and divided into representative samples which were packaged in pliofilm and stored in the freezer. All samples were made up of mixed light, amber, and dark kernels.

The proximate analysis and vitamin determinations were done by the methods described by Hall, Morgan, and Wheeler *(4)*. The fat content was expressed as total petroleum ether soluble content of the dry pulverized samples.

The thiochrome procedure, a combination of the Hennessey and Cerecedo *(5)* and Conner and Straub *(3)* methods, was used for thiamine with omission of the heating before the enzyme digestion with clarase. This produced a more rapid filtering, clearer filtrate than was found when the heating was done. The usual microbiological procedures were used for riboflavin and niacin. There was, however, a change in the procedure used from that described in the previous publication *(8)* in that the samples were prepared for extraction of niacin by heating them for 30 min. at 15 lbs. pressure. In the earlier study no heating had been done. It was found by parallel extractions that without the heating considerably lower niacin values were found in the extracts. The process which yielded the larger niacin values was adopted.

The extraction of pantothenic acid was carried out by the method of Ives and Strong *(6)* and the microbiological procedure followed that of Skeggs and Wright *(14)*. The digestion method of Neilands and Strong *(11)* employing a mixture of alkaline phosphatase and chicken liver enzyme was tried but was discarded because of the varying results obtained. A bioassay was also used, using rat growth as criterion of pantothenic acid content *(4)*.

Folic acid was determined by the microbiological method of Bird *et al. (2)* using hog kidney enzyme for digestion and *S. faecalis* No. 8043 as test organism. The chicken pancreas enzyme method of Mims and Laskowski *(10)* was also used and good agreement obtained between the results of the 2 procedures.

Vitamin B_6 (pyridoxine) was determined by the microbiological method of Atkin *et al. (1)*. Bioassays were made in this case also. The same procedure described previously for thiamine by Jentsch and Morgan *(8)* was followed except that pyridoxine was omitted and thiamine supplied in the deficient vitamin supplement.

[a] The California Walnut Growers' Association, Los Angeles, California, and Mr. Arthur W. Christie kindly secured, stored, and shipped all the samples.

RESULTS

The results of these analyses are shown in Tables 1 and 2. Each value quoted is the mean of 6 to 12 separate and closely agreeing assays. The thiamine and riboflavin determinations on the 1950 crop samples agreed favorably with those found in the earlier experiment (Table 2). The niacin values were in all cases significantly higher than those found in the earlier study. The improved method of extraction accounted for the difference. The niacin content of walnuts thus found agrees more closely with those

TABLE 1

Proximate composition of walnuts

Variety and crop year	Moisture	Ash	Protein	Fat
Payne	%	%	%	%
1950	3.9	1.75	16.2	69.1
1951	3.5	.....	.....	65.2
Placentia				
1950	3.6	1.79	14.9	70.1
1951	3.3	.....	.....	66.9
Franquette				
1950	5.2	1.71	14.8	70.4
1951	3.4	.....	.....	68.2

TABLE 2

B vitamin content of walnuts

Variety and crop year	Thiamin	Riboflavin	Niacin	Pantothenic acid	Folic acid	Vitamin B_6
Payne	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*	*mg. %*
1950	0.28	0.17	1.17	0.67	0.20	.....
1951	.....	0.17	1.19	0.95	0.23	1.05
1942-1945[b]	0.31	0.11-0.16	0.73	.....	.....	.....
Placentia						
1950	0.26	0.17	1.23	0.60	0.22	.....
1951	.....	0.17	1.17	0.72	0.23	0.96
1942-1945[b]	0.29	0.13-0.17	0.81	.....	.....	.....
Franquette						
1950	0.24	0.12	0.89	0.49	0.13	.....
1951	.....	0.14	0.86	0.53	0.13	0.87
1942-1945[b]	0.24	0.10-0.12	0.58	.....	.....	.....

[b] Jentsch and Morgan, 1949.

reported by the Food and Drug Administration[c] to the Office of The Quartermaster General than with our earlier analyses. They also compare well with the levels reported by James *(7)* of 1.44 and 0.78 mg. % in 2 varieties of British-grown walnuts.

The pantothenic acid values of the Payne and Placentia nuts of the 1951 crop were significantly higher than those of the 1950 crop. The Franquettes showed a similar but less striking increase. This is the first crop year effect on the vitamin content of walnuts so far recorded. The only comparable analysis available is that of Jukes *(9)* on English wal-

[c] Personal communication from Dr. E. M. Nelson.

nuts, variety not stated, obtained by the chick growth method, 0.8 mg. %. James *(7)* using a microbiological method reported 0.34 and 0.60 mg. % pantothenic acid in 2 varieties of British-grown walnuts.

As shown in Table 3 the rat growth method yielded the value 1.09 mg. % for 1950 Payne walnuts. Bioassays with either chicks or rats are likely to provide more reliable values for pantothenic acid than the microbiological procedures because of the more complete extraction of the vitamin from its co-enzyme A and other bound forms.

TABLE 3

Comparison of microbiological and bioassays of walnuts

Variety and crop year	Vitamin	Bioassay	Microbiological assay	
				% of bioassay value
Payne		*mg. %*	*mg. %*	
1951	Vitamin B_6	1.08	1.05	97
1950	Pantothenic acid	1.09	0.67	62
Franquette				
1951	Vitamin B_6	0.83	0.87	104

The folic acid content of the Payne and Placentia nuts was the same, 0.20 to 0.23 mg. %. The Franquette variety as usual had a lower value, 0.13 mg. %. The only comparable analysis is that of Toepfer *et al. (12)*, 0.08 mg. % in English walnuts purchased in the Washington, D. C. market. The folic acid levels found in the California-grown walnuts are higher than those of most vegetable foods but are comparable with those of most dried beans, some green leafy vegetables and some meats *(12)*. The high content of solids, about 96%, in these nuts may account for their relatively high levels of folic acid as well as of other B vitamins.

Vitamin B_6 was present in concentrations of 1.05, 0.96, and 0.87 mg. % in the Payne, Placentia, and Franquette walnuts. These values were confirmed by the close agreement found in the bioassays (Table 3). The vitamin B_6 microbiological and bioassays yielded the closest agreement yet found in such comparisons, 97 and 104% of the microbiological values being found by rat growth assay. These levels compare with those reported for wheat germ and soybean flour by Sarma *et al. (13)* but are higher than most of the other values reported for vegetable and some animal foods. Again the concentration of the nuts may account for the difference in ranges reported.

Variety Differences. In all cases the Franquette nuts had lower levels of B vitamin content than did the Placentia and Payne varieties. There was only 60 to 87% of the vitamin values of the latter varieties found in the Franquette walnuts. No corresponding differences in proximate composition were seen. These differences carried over from one crop year to the next even when a change in level occurred as in the case of pantothenic acid in the 1951 samples.

SUMMARY

Walnuts of the Payne, Placentia, and Franquette varieties of the 1950 and 1951 crops and of similar origin to those previously studied were examined for the presence of 6 of the B vitamins. Chemical and microbiological methods were used for the most part but a few rat growth assays were included.

Thiamine was found to be in nearly the same concentrations found earlier, 0.24 to 0.28 mg. %. Riboflavin values similarly agreed, 0.12 to 0.17 mg. %. Niacin, extracted by a more effective method, was found in 40 to 60 % larger amount than in the 1949 analyses. The new levels were 0.86 to 1.23 mg. %. Pantothenic acid by the microbiological method appeared to be present as 0.49 to 0.95 mg. % but by rat growth in one variety 1.09 mg. %. Folic acid was present as 0.13 to 0.23 mg. % and vitamin B_6, 0.87 to 1.05. In the latter case bioassay of 2 varieties confirmed the results.

No effect of crop year was noted except in pantothenic acid of which all the 1951 samples yielded higher values than did those of the 1950 crop. The Payne and Placentia nuts had significantly higher contents of all 6 vitamins for every sample than did the Franquette nuts.

LITERATURE CITED

1. Atkin, L., Schultz, A. S., Williams, W. L., and Frey, C. N. Yeast microbiological methods for the determination of vitamins. Pyridoxine. *Ind. Eng. Chem., Anal. Ed.*, **15**, 141-144 (1943).
2. Bird, O. D., Bressler, B., Brown, R. A., Campbell, C. J., and Emmett, A. D. The microbiological assay of vitamin B_6 conjugate. *J. Biol. Chem.*, **159**, 631-636 (1945).
3. Conner, R. T., and Straub, G. J. Determination of thiamine by the thiochrome reaction. *Ind. Eng. Chem., Anal. Ed.*, **13**, 380-385 (1941).
4. Hall, A. P., Wheeler, P., and Morgan, A. F. The amounts of six B vitamins in fresh and dried figs. *Food Research*, **18**, 206-216 (1953).
5. Hennessy, D. J., and Cerecedo, L. R. The determination of free and phosphorylated thiamine by a modified thiochrome assay. *J. Am. Chem. Soc.*, **61**, 179-183 (1939).
6. Ives, M., and Strong, F. M. Preparation of samples for the microbiological assay of pantothenic acid. *Arch. Biochem.*, **9**, 251-258 (1946).
7. James, D. P. Nicotinic acid, pantothenic acid and biotin in fruits, vegetables and nuts. *Brit. J. Nutrition*, **6**, 341-356 (1952).
8. Jentsch, M. S., and Morgan, A. F. Thiamin, riboflavin and niacin content of walnuts. *Food Research*, **14**, 40-53 (1949).
9. Jukes, T. H. The distribution of pantothenic acid in certain products of natural origin. *J. Nutrition*, **21**, 193-200 (1941).
10. Mims, V., and Laskowski, M. Studies of vitamin B_6 conjugate from chicken pancreas. *J. Biol. Chem.*, **160**, 493-503 (1945).
11. Neilands, J. B., and Strong, F. M. The enzymatic liberation of pantothenic acid. *Arch. Biochem.*, **19**, 287-291 (1948).
12. Toepfer, E. W., Zook, E. G., Orr, M. L., and Richardson, L. R. Folic acid content of foods. U. S. Dept. Agr. *Handbook*, **29** (1951).
13. Sarma, P. S., Snell, E. E., and Elvehjem, C. A. The bioassay of vitamin B_6 in natural materials. *J. Nutrition*, **33**, 121-128 (1947).
14. Skeggs, H. R., and Wright, L. C. The use of *Lactobacillus arabinosus* in the microbiological determination of pantothenic acid. *J. Biol. Chem.*, **156**, 21-26 (1944).

A DEVICE FOR THE QUANTITATIVE INOCULATION OF SURFACES WITH BACTERIAL CELLS: DESIGN AND PERFORMANCE[a,b]

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Epidemiological evidence points to surface contamination from the nose and throat as a common means of transmission in outbreaks of food poisoning traced to micrococci. As a part of a study which attacked this general problem, it was necessary to devise a method for the quantitative inoculation of surfaces with bacterial cells. Spray inoculation of food samples is not a new technique. Scott *(1)* and Segalove and Dack *(2)* are among those who have used this method. No reference was found, however, to attempts to make quantitative use of such a technique. This paper describes an apparatus which proved satisfactory for this purpose. Performance records are presented. The paper is offered with the hope that the apparatus or some modification of it may be found useful in other lines of investigation.

DESIGN AND CONSTRUCTION

The design here described was adapted from one used by Waters *(1)* in an investigation of lethal concentrations of insecticides. The apparatus consisted essentially of an atomizer through which a suspension of bacterial cells was sprayed on the sample. Figure 1 shows details of construction.

The upper cylinder of the apparatus was formed from a sheet of cellulose acetate[c] 25 in. by 40 in., the edges of which were overlapped ⅛ in. and sealed with acetone. A sheet of hardware cloth formed a shelf for supporting this cylinder and provided a place for the food sample. Contamination of the air in the room by the cells of the inoculum was minimized by placing excelsior wet with a bactericidal agent in the lower cylinder. Space was allowed for the free passage of air currents through the 2 cylinders. Such an arrangement was necessary in order to prevent the building up of pressure which would have interferred with the uniformity with which the cells fell on the sample.

The glass atomizer (Figure 1-E) was constructed so that it was capable of producing a fine mist when pressure was supplied by compressed nitrogen. The nitrogen and the inoculum inlet tubes were formed from Pyrex tubing. Approximate aperture sizes were obtained by cutting off the drawn ends of the tubes and shrinking the apertures in the flame until wire of appropriate gauge just fitted into the opening. Twenty-gauge wire (0.8 mm. diameter) and 19-gauge wire (0.9 mm. diameter) were used for the liquid inlet tube and the nitrogen tube respectively. (See Figures 1-B and 1-D.)

The nitrogen and inoculum inlet tubes were spaced with a basswood block (Figure 1-C). Adjustment was accomplished by binding the 2 tubes to the wood block loosely and manipulating their relative positions so that the blast of nitrogen created a vacuum and drew the inoculum up into the path of the stream of nitrogen where it was broken

[a] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison, Wisconsin.

[b] Supported in part by a grant from Swift and Company, Inc., Chicago, Illinois.

[c] Manufactured by E. T. Keeler and Co., 500 N. Dearborn Street, Chicago, Illinois.

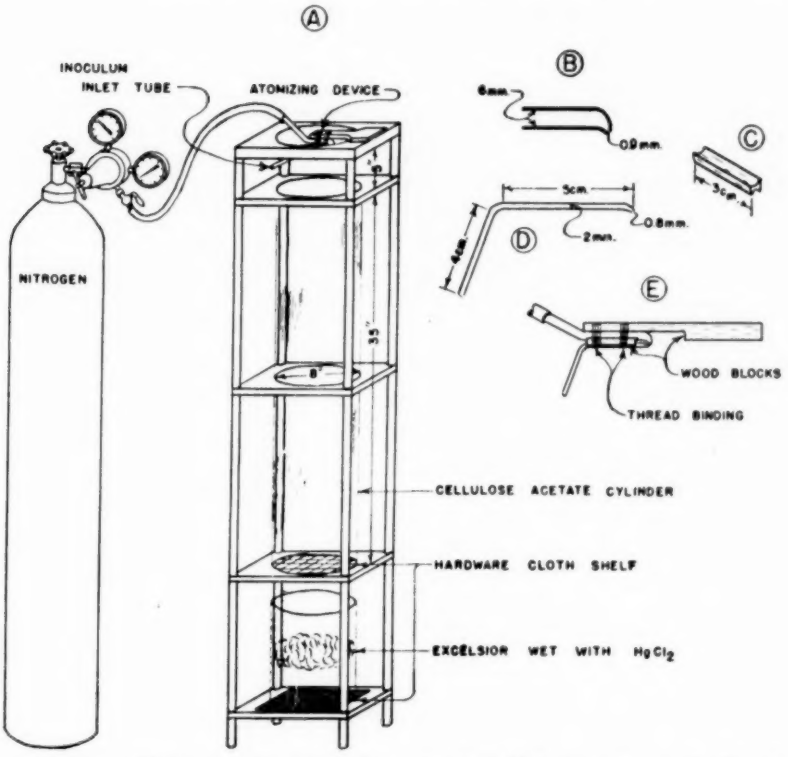

Figure 1. Diagram of spray equipment: A—Assembled spray tower; B—Tip of nitrogen tube; C — Wood spacing block; D — Inoculum inlet tube; E — Assembled atomizer.

into fine droplets. The atomizer adjustment was conveniently tested by spraying a dye solution on unglazed white paper. When a broad even spray was obtained the tubes were bound firmly with fine strong thread and retested. Rigidity and permanence were given to the adjustment by painting the thread binding with several coats of a cement made of cellulose acetate dissolved in acetone. The assembled atomizer was then bound to a wooden block (Figure 1-E) and attached to the frame of the spray tower in such a position that the spray was directed down the center of the cylinder.

A satisfactory inoculation resulted when nitrogen at a pressure of 10 pounds was used to atomize one ml. of inoculum. Regulation of the pressure was accomplished by fitting a standard nitrogen cylinder with a reduction valve and gauge. A gas cock welded to the outlet provided a simple means of manipulation without disturbing the pressure adjustment.

The inoculum was prepared by suspending the cells from a 24-hour culture of *Micrococcus pyogenes* var. *aureus* in saline. The suspension was shaken with glass beads and strained through sterile cotton to remove clumps of cells and agar particles. Concentration was regulated by further dilution with saline to give a reading of 10% light transmission in a Lumetron photoelectric colorimeter. Such suspensions were found to contain approximately 2.5 billion cells per ml. The inoculum was used in this form or diluted as needed.

STANDARDIZATION AND PERFORMANCE

Preliminary tests of performance were made using dye solution as the liquid. When the color of the paper receiving the spray was quite uniform, it was assumed that a uniform dispersion of droplets was being produced. Tests were then made in which suspensions of bacterial cells were sprayed on ham samples 5 cm. by 5 cm. square.

TABLE 1

Cells of Micrococcus pyogenes var. aureus deposited on ham samples by spray inoculation (inoculum = approximately 25×10^7 cells/ml.)

Test I			Test II		
Day	Sample	Cells per sample	Day	Sample	Cells per sample
		$\times 10^5$			$\times 10^5$
1	1	655	1	1	77.7
	2	510		2	58.6
	3	520		3	99.8
	4	850			
	5	465	2	4	134.5
	6	515		5	87.4
				6	44.9
2	7	520			
	8	360	3	7	120.9
	9	840		8	82.1
	10	420		9	84.4
	11	615	4	10	92.0
	12	430		11	85.2
				12	100.0
3	13	740			
	14	705	5	13	130.3
	15	480		14	117.9
	16	675		15	105.2
	17	675			
	18	605			
Mean		588			94.7
Standard deviation		142			24.9
Relative standard deviation		24%			26.6%

Results of a typical test are shown in Table 1, Test I. The data for Test II of this table are presented for comparison. The second test was made 6 months after the first one and subsequent to dismantling and reassembling the atomizer. It will be noted that while a change in atomizer adjustment produced a marked difference in the number of cells deposited on the sample, there was close agreement in the relative standard deviation of the two tests.

Although the performance of the apparatus with a given adjustment of the atomizer seemed relatively satisfactory, certain questions presented themselves. It appeared desirable to investigate the effect of the concentration of the inoculum on performance and to study the results obtained with more than one type of sample. An experiment was designed which permitted the simultaneous comparison of these two variables.

Inocula which contained approximately 25×10^7 and 25×10^6 cells per ml. were used to inoculate samples of ham and samples of sterile bibulous paper. Six replications were made of each phase of the procedure. Results are shown in Table 2 and Table 3.

DISCUSSION

The spray device described was shown to be capable of producing surface inoculations which were sufficiently uniform to be useful in certain types of quantitative bacteriological work. Changes in the adjustment of

the atomizer parts affected the number of cells deposited. It seemed desirable therefore to maintain the adjustment undisturbed within any given experiment.

It is often desirable in bacteriological studies to compare results obtained with varied levels of inoculation. In such work it is necessary to adjust the inoculation to approximate a predetermined number of cells. Data presented in Table 2 indicate the extent to which the spray apparatus

TABLE 2

Cells of Micrococcus pyogenes var. aureus deposited on ham samples as affected by concentration of inoculum

Day No.	Inoculum 25×10^7 cells/ml.	Day No.	Inoculum 25×10^6 cells/ml.
	$\times 10^5$		$\times 10^5$
1	777	1	86
	586		79
	998		74
2	1345	2	67
	874		71
	449		98
		3	85
			100
			95
3	1209	4	104
	821		89
	844		63
4	920	5	93
	852		69
	1000		81
5	1303	6	62
	1179		122
	1052		94
Mean	947		85
Standard deviation	249		16
Relative standard deviation	26.6%		18.8%

is useful in such a problem. It was to be expected that dilution of the inoculum would affect the number of cells deposited. When the inoculum was diluted tenfold, the number of cells deposited was decreased in roughly the same manner. It would therefore appear that the use of dilutions of an inoculum of known cell concentration would afford a method for arriving at a desired level of inoculation so long as the atomizer adjustment remained unchanged.

The data presented in Table 3 show results obtained when ham and paper samples were subjected to the same inoculating technique. These data were subjected to an analysis of variance for completely randomized experiments. Logarithms of the numbers of cells were used. The variances of the logs were then compared simultaneously for homogeneity by Bartlett's test [Snedecor *(3)* p. 250]. No significant differences were indicated.

TABLE 3

Atomizer performance at two levels of inoculation for two types of samples
Mean of at least 15 trials

	Inoculum = approximately 25×10^7 cells/ml.	Inoculum = approximately 25×10^6 cells/ml.
	Mean no. cells $\times 10^4$	*Mean no. cells* $\times 10^4$
Ham	947	85
Paper	286	24

This fact establishes that satisfactory performance is independent of the level of inoculation. Secondly, it indicates that while a markedly smaller number of cells was recovered from paper samples as compared to ham samples, results are consistent within sample type. This would lead one to assume that the dispersion of droplets of inoculum by the atomizer was relatively uniform. The only explanation that is offered for the low values reported for the cells deposited on paper is that absorbency of the paper may have prevented cell recovery.

In the work described here the primary concern has been with the quantitative performance of the device described. Little attention has been given to the problem of aerosol production or containment. In work with pathogens this would of course be a factor of importance; it is, however, outside the scope of this paper.

SUMMARY

The construction and operation of equipment for spray inoculation has been described. Tests with this equipment have shown it to be capable of reproducible results within limits when used with standardized cell suspensions of various concentrations. The level of inoculation achieved on a surface appears to be influenced by concentration of inoculum, by the atomizer adjustment, and by the nature of the surface of the sample.

Acknowledgment

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LITERATURE CITED

1. Scott, W. J. The growth of micro-organisms on ox muscle. II. The influence of temperature. *Australia J. Council Sci. Ind. Research*, **10**, 338 (1937).
2. Segalove, M., and Dack, G. M. Growth of bacteria associated with food poisoning experimentally inoculated into dehydrated meat. *Food Research*, **16**, 118 (1951).
3. Snedecor, G. W. *Statistical Methods.* 4th Ed., 1946, The Iowa State College Press, Ames, Iowa.
4. Waters, H. A. General methods and equipment. *Laboratory Procedures in Studies of the Chemical Control of Insects.* Publication No. 20, American Association for the Advancement of Science, Washington, D. C. (1943).

A TECHNIQUE FOR DETECTING FLAVOR CHANGES IN STORED DRIED EGGS[a]

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During World War II considerable emphasis was placed on improving the shelf life of many dehydrated foodstuffs, including dried eggs. Workers studying dried eggs were concerned with losses of nutritive elements and deterioration in physical properties and palatability. Of most practical importance were probably those changes affecting palatability, since the nutritional values of a product cannot be realized unless the product is consumed.

The complex of factors affecting palatability including color, texture, taste, and odor, makes it a difficult variable to measure accurately. A chemical method in which the fluorescence of a saline extract of the defatted powder is determined, has been presented by Pearce and Thistle *(14)*. Results of this method are correlated with panel-determined palatability according to Pearce, Reid, and Cook *(13)*, Marcus *(11)*, and Pearce and Thistle *(15)*. The results of the ether-soluble fluorescence method developed by Dutton and Edwards *(7)* are even more closely related to palatability, especially in low moisture powders, according to Boggs, Dutton, Edwards, and Fevold *(3)*. Another chemical method for determining palatability has been proposed by Steffen, Hopkins, Kline, and Whetzell *(17)* which also seems to be correlated with palatability. Present chemical tests, however, do not consider all factors affecting palatability and may well be measuring changes not affecting palatability directly. Therefore, while they are useful laboratory tools, they are seldom used alone as critical tests of palatability.

Most palatability determinations on dried eggs have been made by a selected panel of judges who score the eggs from very good (score of 10) to very poor or inedible (score of 0). Several laboratories using large or highly selected panels have reported good results by this method (Levin, *10*). Such tests are at least partially subjective since they measure flavor preference as well as actual deviation from a standard considered acceptable.

In a study in which the chief objective is to determine the degree of change which occurs in dried eggs prepared and stored under different conditions for various periods of time, it is essential to have a method of palatability testing whereby reliable comparisons can be made between the scores of the original sample and the samples tested after storage. In

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the present study, a group of 20 girls from the clerical staffs was tested for sensitivity to the four primary flavors and then trained as judges to score dried egg samples. After considerable time and effort had been expended, it became obvious that a consistent and reliable scoring panel could not be developed from the relatively small number of candidates available. Consequently, an effort was made to develop a more objective technique, adaptable to situations where but few judges were available. Several procedures were tried, and the method finally developed has been briefly described by Bohren and Jordan *(4)* and Boggs and Hanson *(2)*.

METHOD

The technique developed consists of diluting the experimental dried eggs into fresh shell egg to the point where the mixture is just distinguishable after cooking from the undiluted fresh egg control. Thus, the scoring is based on the flavor threshold of the tasters rather than on their judgment. The object of the test therefore, is to detect differences rather than to determine which sample is preferred. Applications of this principle have been made by Fair *(8)* in studying odors in water, and Crocker *(6)* and Trout and Sharp *(17)* in taste studies on solutions of the primary flavors. It was also used in the development of sound intensity units, the bel and decibel (see Colby, *5*). Tests conducted on panel members indicated that 20% decreases in amount of experimental egg in the control fresh egg were consistently detected. This figure compares with 6-10% for tasting pure solutions and 15-20% for odor difference perception reported by Crocker *(6)*. Trout and Sharp *(18)* found that 5-10% changes in dilutions of the primary flavors could be detected depending upon the type of flavor being studied.

According to the Weber-Fechner law, the intensity of a sensation varies with the logarithm of the intensity of the stimulus. This principle has been utilized in the development of sound intensity measurement and the same principle was used in establishing scores for flavor distinction in dried eggs as follows:

Taste units (score)	Amount of reconstituted dried egg in fresh egg (%)
0	100.0
1	79.5
2	63.1
3	50.1
4	39.8
5	31.6
6	25.1
7	20.0
8	15.8
9	12.6
10	10.0

In this case arithmetic changes in sensation level (score) represent logarithmic changes in stimulus (percent dilution). The dilutions for scores from 11 to 20 would be an exact duplication of the series from one to 10, except that in each case the dilution would be one-tenth as much in the second series as in the first. A difference of 10 units in taste score thus means a ten-fold difference in dilution percentage.

In a technique of this design, a constant control must be used. In the present case, fresh eggs were the obvious answer. However, detectable variation exists between fresh eggs from different sources, probably due to breed, or diet of the hens, or to the age of the eggs. Therefore, a small group of birds of one breed which was fed the same diet was maintained to produce the control fresh eggs for this experiment. Only eggs laid the day previous to a trial and held under refrigeration after laying were used.

Since the dried eggs stored at high temperatures were often dark in color, they could be identified even at low dilutions by color alone. In order to eliminate this variable, all samples tasted were artificially dyed to the same shade with a tasteless, odorless dye. Commercial liquid food colors were found to be unsatisfactory for this purpose because of the presence of preservatives such as tartaric or citric acid. However, certified dry coal tar food dyes were satisfactory. For this experiment, straw-

berry red (a mixed ice cream color), was used. A similar camouflage procedure was utilized by Moir *(12)* in studying chocolate and vanilla flavors in sponge biscuits.

Only one sample of dried egg was studied at any one panel session. The fresh eggs were broken out and thoroughly mixed. The dried egg sample to be tested was reconstituted by adding 90 ml. of distilled water to 30 g. of egg powder. Different amounts of the reconstituted dried egg were weighed into six 250 ml. beakers. The amounts were determined by the six consecutive scoring dilution rates which were estimated to bracket the 75% correct point. Each beaker was then brought to 100 g. by the addition of mixed fresh egg. To these 6 beakers as well as to an equal number containing 100 g. of fresh egg for the controls, was added a measured amount of the strawberry ice cream coloring. All beakers were then placed in a boiling water bath, and the contents stirred until complete coagulation occurred.

For presentation to the tasters, the eggs were placed in petri dishes on metal trays. Two trays were prepared for each dilution level and each tray contained 10 petri dishes. On each tray one dish was identified as containing fresh egg, while a second was identified as containing the experimental diluted dried egg. The other 8 dishes on each tray were identified only by numbers from one to 8, and contained either the fresh undiluted or the experimental egg. The distribution of the samples to these 8 unknown dishes was at random so that no regular sequence or number of samples could be utilized in identification. The tasters merely reported the 8 unknown samples as fresh or experimental egg. Since 6 different dilution levels were used in each test, 12 such trays were prepared.

The eggs were allowed to cool to room temperature before being served to the panel. The odor was greater at higher temperatures and tended to affect the scoring. Therefore, it was necessary to have all samples at the same temperature when scored. In the absence of any equipment to maintain a constant high temperature, room temperature was used and gave good results.

The taste panel consisted of 6 girls selected for low sensation threshold to the flavors in stored dried eggs. Since each taster made 16 observations at each dilution level, a total of 96 judgments were available for each of the 6 dilutions. The percentages of correct judgments at each dilution level were plotted against the dilution percentages or scores (Figure 1). From these data, a regression line was plotted by the method of least squares. A perpendicular line was drawn from the point where the regression line intersected the 75% correct judgment horizontal line. The score or sensation level of the sample was then read from the bottom of the graph.

For this method to be accurate, it is necessary for the percentages of correct judgments for the different dilution rates to be fairly evenly distributed above and below the 75% correct level. For this central region of the curve, a linear relationship was found to exist between the 2 variables (see Table 1). However, as the 50% or 100% correct judgment levels are approached, the regression becomes curvilinear and of smaller magnitude as is typical of a sigmoid curve. The points where this effect makes its appearance were not determined. With the small number of data involved, the sampling error of the regression coefficients is somewhat large and the average of a

TABLE 1

Analysis of variance of results of testing the same dried egg sample on different days by an experienced and an inexperienced taste panel

Source of variation	df.	Experienced panel			Inexperienced panel		
		S.S.	M.S.	F.	S.S.	M.S.	F.
Days	6	1979.81	329.97	8.12**	398.90	66.48	1.20
Dilution levels	5	3557.21	711.44	8.68**	2906.39	581.28	17.69**
Linear regression	1	3223.37	3223.37	.....	2831.89	2831.89	.....
Deviations from linear reg.	4	333.80	83.45	1.02	74.53	18.63	0.57
Days × dilutions	30	2459.26	81.98	2.02*	985.79	32.86	0.59
Error	42	1707.75	40.66	.....	2332.64	55.54	.....

* Significant, $p = <.05$.
** Highly significant, $p = <.01$.

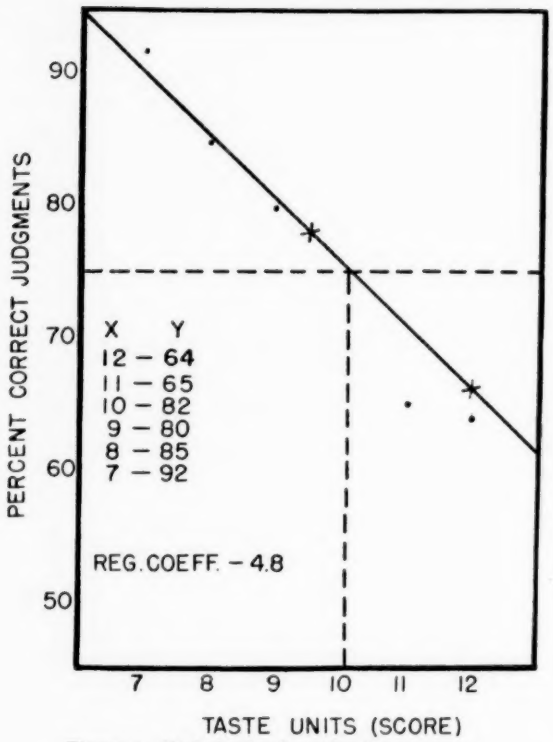

Figure 1. Method of calculating flavor scores.

large number of regression coefficients should be used in calculating the score. In this study, the average of 27 different estimates of the regression coefficient was —4.8. The values for the regression coefficients were found to be unrelated to the score of the

TABLE 2

Repeated evaluations of a single dried egg sample by a panel of six experienced tasters

Day No.	Score		Regression coefficient
	By average regression coefficient	By calculated regression coefficient	
1	11.8	12.0	—3.8**
2	9.9	10.0	—5.8*
3	11.8	12.0	—3.9
4	12.1	12.2	—4.6**
5	10.5	10.5	—4.4*
6	12.1	11.9	—5.3**
7	12.2	11.9	—5.5*
Average	11.5	11.5	—4.76
Standard deviation	±.92	±.87	± .83

* Significant, $p = < .05$.
** Highly significant, $p = < .01$

samples tested. It is further evident that if the mean values are near the 75% correct horizontal line, variations in the regression coefficient will have only a small influence on the estimated score.

Some refinements of this method are possible which would straighten the tails of the sigmoid curve. This would increase the region of linearity of the curve and reduce the necessity for having the average percent correct judgments near the 75% correct level. To accomplish this it is first necessary to expand the range of the percent correct (Y) scale from 50-100%, to read 0-100%. The equation $p = 2(j-50)$ where p = the percentage for the 0-100% scale, and j = the raw percentage or that on the 50-100% scale, is suitable for this purpose. The p values can then be transformed to probits (Finney, 9). Plotting the percent correct judgments as transformed to probits (Y') with the scores or dilution levels (X) would result in essentially a straight line.

TESTS OF RELIABILITY

To test the reliability of the technique, the 6 experienced and sensitive tasters were used to score the same dried egg sample at the same dilutions on seven different days. The results of this test are shown in Table 2. The data from this test were transformed from percentages to arc sines and subjected to analysis of variance (Table 1). For the purpose of this experiment, days were considered to be a random variable and their variance was tested for significance by the residual error variance. Dilution levels however were considered to be fixed within the limits of this experiment and their variance tested for significance by the days × dilution interaction variance. It is evident from this analysis that highly significant differences between dilution levels exist. Furthermore it is demonstrated that almost all of the differences between dilution levels were accounted for by the linear regression since the deviations from linearity were small and non-significant. Of more importance is the fact that highly significant differences occurred between days, indicating that the tasters were not consistent from day to day in their total accuracy over all dilutions. However, when translated into scores as shown in Table 2 the actual variation is apparently small.

As indicated above, this technique is based on flavor thresholds rather than flavor judgment. Since flavor thresholds are considered to be constant for each individual it was postulated that an inexperienced panel should give results as reliable as those produced by a panel specially selected for taste sensitivity, but that the scores should be lower. Such inexperienced tasters would be expected to be erratic until they had become familiar with the flavors they were supposed to detect. A panel of 6 girls who had never tasted dried eggs or served on any taste panel before was established. For this inexperienced panel it was found that on 5 succeeding days the scores for a single sample became progressively higher. The regression also failed to be significantly different from zero in three of the five tests. These results are shown in Table 3.

After this preliminary period for learning to recognize the flavor, the same 6 inexperienced girls on 7 different days scored the same dried egg sample as that previ-

TABLE 3

Preliminary evaluations of a single dried egg sample by a panel of six inexperienced tasters

Day No.	Score		Regression coefficient
	By average regression coefficient	By calculated regression coefficient	
1	10.9	11.7	−1.59
2	13.2	13.9	−3.47*
3	13.8	14.7	−3.06**
4	14.8	16.2	−2.35
5	15.2	15.9	−3.41
Average	13.6	14.5	−2.78

* Significant, $p = < .05$.
** Highly significant, $p = < .01$.

ously scored by the experienced panel. The results are shown in Table 4. It is evident that the average score, 9.4, is much lower than the 11.5 score obtained by the experienced panel. The reliability as indicated by a standard deviation of ±.71 is equally as good as that of the experienced panel. A summary of the analysis of these data in a manner similar to that for the data from the experienced panel is shown in Table 1. Highly significant differences between dilution levels are evident. This can again be attributed primarily to the regression of percent correct judgments on percent dilution (score) since the deviations from linearity did not approach significance. The differences between days were not significant indicating that this panel was consistent in its daily accuracy.

These data show that while the reliabilities of the 2 panels were about equal, the taste thresholds for the 2 were quite different. This difference between the panels reflects individual differences in taste perception, possibly of genetic origin (Salmon and Blakeslee, *16;* Blakeslee and Salmon, *1*). Such individual variability necessitates the use of the same tasters for scoring any series of samples which are to be compared.

TABLE 4

Repeated evaluations of a single dried egg sample by a panel of six inexperienced tasters

Day No.	Score		Regression coefficient
	By average regression coefficient of −4.8	By calculated regression coefficient	
1	9.1	8.7	−3.80*
2	9.7	9.7	−4.59*
3	10.1	10.0	−5.82**
4	8.8	9.0	−6.17*
5	9.1	9.0	−3.88
6	9.1	9.1	−5.76**
7	10.5	10.2	−6.76**
Average	9.4	9.4	−5.25
Standard deviation	±.71	±.62	±1.18

* Significant, $p = < .05$.
** Highly significant, $p = < .01$.

TESTS FOR TASTE FATIGUE

Each taster made 96 different judgments in the course of less than an hour. This raises the question as to whether or not taste fatigue might affect the results by raising the taste threshold, and consequently affecting the scores. In order to estimate the effect of taste fatigue upon the taste threshold, a single sample of dried eggs was prepared at 2 dilution levels, one known from previous tests to be detectable in more than 75% of the judgments and another which the panel had previously been unable to detect 75% of the time. The two dilutions and controls were processed and presented to a 5- and 4-girl experienced panel respectively, on 2 different days. The correct judgments by the tasters were totaled according to the order of tasting the 12 series. Thus, the correct judgments for the first to the twelfth trays tasted by each taster can be compared. The results are shown in Figure 2. For analysis of variance each 2 consecutively tasted trays were combined so that the procedure would conform to that actually used in testing experimental samples. This also allows calculation of an error term which increases the accuracy of the tests of significance and makes possible a test for interaction. Results of analysis by individual trays, however, did not differ from those from the study of paired trays. Results of both methods of analysis are shown in Table 5.

It is evident that no significant differences exist in the numbers of correct judgments between individual trays or pairs of trays. Thus the fluctuations in correct judgments as seen in Figure 2 are due to chance and it may be concluded that in this method, taste fatigue does not affect the scoring of test samples, when no more than

96 are judged at one session. The highly significant differences between tasters again illustrates the individual differences in taste threshold and emphasizes the necessity of using the same tasters in scoring any samples which are to be compared.

DISCUSSION

Organoleptic tests may be generally classified as subjective since decisions involve the application of judgment by the taster. This judgment also involves flavor preference. The purpose of the present study was to study flavor change rather than preference; hence the technique described here is based on taste perception, a biological and more or less constant entity for each individual. By using taste perception as an evaluating instrument and sufficient comparisons to give statistical validity, a near approach to an objective biological test is achieved.

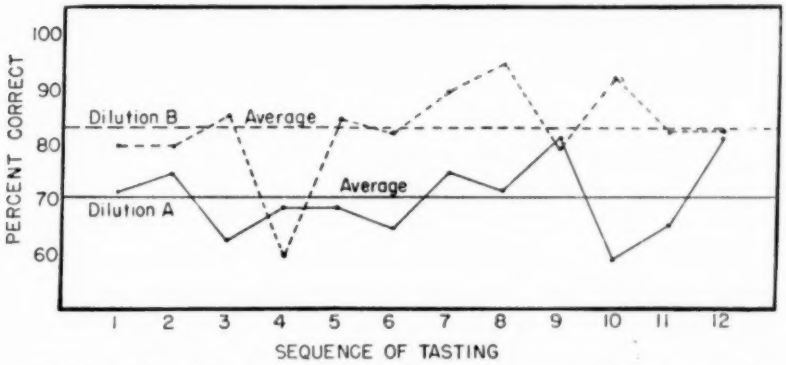

Figure 2. The effect of taste fatigue. The percent correct judgments in chronological sequence on twelve trays of dried eggs at each of two dilutions, showing variation around the averages.

It is evident from this study that different panels may give different scores to the same egg even when the same control egg is used. Since the important consideration is flavor difference, the absolute score of a given egg is of relatively minor interest. No comparative data are available but it would appear that the 2 panels would have given similar results if the amount of flavor difference between 2 experimental samples were considered. Differences between samples would also be comparable between different laboratories although the absolute scores might be different, both due to panels and the controls used.

Since a panel unselected as to sensitivity gave equally as good results as a highly sensitive panel, the utilization of an unselected panel would be preferred because of other advantages. Unselected panels at different laboratories would be expected to be more nearly equal since both would approach the mean of the population in perceptivity. Furthermore, while it is undesirable to change panel members during a series of tests, if it became necessary to replace a panel member, the substitute member would probably not deviate greatly from the mean sensitivity of the panel. Sat-

isfactory substitutes in highly sensitive panels are difficult to find. While only 6 tasters were utilized in the present study, the number of tasters could be increased on an unselected panel which would add to the precision of the test without greatly increasing the labor involved in making the test.

TABLE 5

Analysis of variance of two sets of 12 trays of eggs with identical dilution levels arranged in sequence of tasting

(a) Analysis by consecutively tasted pairs of trays

Source of variation	Dilution A				Dilution B			
	df.	S.S.	M.S.	F.	df.	S.S.	M.S.	F.
Pairs of trays	5	603.6	120.7	0.96	5	2023.5	404.7	0.73
Tasters	3	3372.40	1124.1	5.16**	4	6999.2	1749.8	5.91[1]
Series × tasters	15	1891.2	126.1	0.58	20	11048.9	552.4	1.87
Error	24	5232.7	218.0	.....	30	8880.9	296.0	.....

(b) Analysis by individual trays

Trays	11	1665.5	151.4	0.82	11	3516.7	319.7	0.76
Tasters	3	3372.4	1124.1	6.12**	4	6999.2	1749.8	4.18[1]
Trays × tasters	33	6062.0	183.7	.....	44	18436.6	419.0	.....

** Highly significant, $p = < .01$.

SUMMARY

In order to study progressive flavor changes in stored dried eggs with a small number of tasters, an objective technique was developed which gives reliable flavor scores.

Six different dilutions of dried egg in fresh egg were scrambled. The percentage dilution was based on a logarithmic scale. Addition of a certified food dye minimized color differences. Two trays of 10 dishes of egg were used at each dilution level. In each tray one dish was a known fresh egg, one a known diluted experimental sample, the other 8 were coded unknowns, and each may have been either fresh, or diluted experimental egg. Six tasters identified each of the 8 unknowns as fresh or experimental. The percentage of correct judgments at each of the 6 dilution levels was then plotted against the percent dilution. A regression line was plotted for the 6 data points. A perpendicular line dropped from the point where the regression line intersects the 75% correct horizontal line determines the score.

This method is adaptable to any food substances which can be made homogeneous and for which adequate constant controls are available. This might apply to such products as potatoes and milk. It would also be adapted to studies concerning the effect of diet and storage conditions on shell eggs and poultry.

Acknowledgment

The authors are grateful for the many suggestions received during the course of this work, and in particular, for those of Dr. E. H. Scofield of Joseph E. Seagram and Sons, Incorporated; Dr. G. F. Stewart, now Chairman of the Division of Poultry Husbandry, University of California; and the late Dr. R. M. Conrad of the University of Denver. The cooperation of the clerical employees serving on the taste panels, without whom this study could not have been conducted, is gratefully acknowledged. Considerable assistance with the statistical analysis was provided by Dr. V. L. Anderson of the Statistics Department, Purdue University.

LITERATURE CITED

1. Blakeslee, A. F., and Salmon, T. N. Genetics of sensory thresholds: Individual taste reactions for different substances. *Proc. Nat. Aca. Sci.*, **21**, 84 (1935).
2. Boggs, Mildred M., and Hanson, Helen L. Analysis of foods by sensory tests. *Advances in Food Research*, II, 219 (1949).
3. Boggs, M. M., Dutton, H. J., Edwards, B. G., and Fevold, H. L. Dehydrated egg powders. Relation of lipide and salt water fluorescence values to palatability. *Ind. Eng. Chem.*, **38**, 1082 (1946).
4. Bohren, B. B., and Jordan, Ruth. An objective technique for detecting flavor changes in dehydrated eggs. *Poultry Sci.*, **25**, 397 (abstract) (1946).
5. Colby, M. Y. *Sound Waves and Acoustics*. 1938, Henry Holt & Co., New York, N. Y.
6. Crocker, E. F. Measuring food flavors. *Food Research*, **2**, 273 (1937).
7. Dutton, H. J., and Edwards, B. G. Changes in stored dried eggs. Spectrophotometric and fluorometric measurement of changes in lipides. *Ind. Eng. Chem.*, **37**, 1123 (1945).
8. Fair, G. M. Determination of odors and tastes in water. *J. N. E. Water Works Assoc.*, **47**, 248 (1933).
9. Finney, D. J. *Probit Analysis*. 1947, Cambridge Univ. Press.
10. Levin, G. Taste scoring tests on dried whole eggs. *U. S. Egg and Poultry Mag.*, **49**, 371 (1943).
11. Marcuse, Sophie. Applying control chart methods to taste testing. *Food Inds.*, **19**, 316 (1947).
12. Moir, H. C. Some observations on the appreciation of flavor in foodstuffs. *J. Soc. Chem. Ind.*, **55**, 145 (1936).
13. Pearce, J. A., Reid, M., and Cook, W. H. Dried whole egg powder. XVIII. The keeping quality of acidulated, gas packed powders of low moisture content. *Can. J. Research*, Sec. F, **24**, 39 (1946).
14. Pearce, J. A., and Thistle, M. W. Fluorescence as a measurement of quality in dried whole egg. *Can. J. Research*, Sec. D, **20**, 276 (1942).
15. Pearce, J. A., and Thistle, M. W. Dried whole egg powder. XXVIII. Reproducibility and interrelation of methods of assessing quality. *Can. J. Research*, Sec. F, **27**, 73 (1949).
16. Salmon, T. N., and Blakeslee, A. F. Genetics of sensory thresholds: variations within single individuals in taste sensitivity for PTC. *Proc. Nat. Acad. Sci.*, **21**, 78 (1935).
17. Steffen, A. H., Hopkins, E. W., Kline, R. W., and Whetzell, G. H. A chemical method for scoring dried whole eggs. *U. S. Egg & Poultry Mag.*, **49**, 308 (1943).
18. Trout, G. M., and Sharp, P. F. The reliability of flavor judgments with special reference to the oxidized flavor of milk. *Memoir 204*, Cornell Univ. Ag. Expt. Sta. (1937).

HISTOLOGICAL INVESTIGATIONS OF TEXTURE IN APPLES. I. COMPOSITION AND INFLUENCE OF HEAT ON STRUCTURE

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Changes from succulence to mealiness during ripening and storage of apples are important to the marketability of the fresh fruit. These changes also are significant to the textural qualities of processed apples. Mealy apples frequently sauce readily when cooked. Some varieties sauce rapidly when cooked in the fresh condition; others remain intact and relatively firm.

It has been commonly accepted that saucing of cooked apples is the direct result of cell separation when the middle *lamella* pectins are solubilized *(7, 12)*. Mealiness, as a condition of cell separation in fresh apples, has long been considered to result from pectic changes during ripening and Branfoot *(1)* demonstrated that these changes, from insoluble "protopectin" to water-soluble pectins, occur in the middle *lamellae* between adjacent cells. Apples vary considerably in textural qualities, however; some of the conditions described by Branfoot do not agree with observations made by Tetley *(11)* and the composition of middle lamellae, *in situ*, still remains conjectural. The exact chemical composition of Branfoot's "protopectin" is obscure *(4, 7)* and the term has little meaning other than its reference to the water-insoluble fraction removed by the Carré-Haynes method *(2)*. The soluble fraction, on the other hand, is extracted from the frozen, finely ground tissue and it would be erroneous to assume that it represents the water-soluble pectins of only the middle lamellae.

Orthodox analytical methods are not apt to clarify the composition of the middle lamella materials, *per se*, because these substances also occur in the cellulosic matrix of plant cell walls and some pectins may be in the cell contents *(3, 7, 12)*. The middle lamellae also are known to contain other materials which are likewise cell-wall-encrusting, such as cutins, suberins, lignins, "hemicelluloses," and proteinaceous materials *(3, 8, 10)*. The extent to which these may be present in apple tissues is not known.

Histological changes concerned with the texture of other fruits and vegetables have been reported by several investigators *(8, 12)*. One of the important changes in the heating of fruit tissues is the expansion and escape of intercellular gases *(12)*. Size of cells, intercellular spaces, and physiological conditions, such as turgor pressure, of the fresh tissues are among the factors which influence textural qualities in fresh tissues and which can also affect texture in processed tissues. Progressive loss in tissue tensile strength has been measured for apples subjected to chemical treatments by Griffin and Kertesz *(4)*. Their results simulate the conditions of cell separation in both the ripening and the cooking of the fruit. Few other detailed studies have been made on the mechanisms of cell sep-

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aration and other cellular changes that may occur during the processing of apples, or on how different structural characteristics may relate to texture.

Investigations on the textural changes of different apples when processed were conducted at this laboratory for 5 seasons (1947-1951). Detailed histological observations, as well as proximate composition and determinations of soluble and insoluble pectins were included in these studies. In the present paper the results of controlled heating tests and of chemical analyses are discussed with reference to the texture of several varieties of western apples. Cell size, cell wall surface, and distribution of intercellular spaces in different apples will be described in a second paper.

MATERIALS AND METHODS

Materials. Delicious, Jonathan, Newtown Pippin, Rome Beauty, and Winesap apples from Washington, Gravenstein and Newtown Pippin from California, and Cortland and McIntosh from eastern states were used for heating experiments on apples of the 1949 season. The Washington apples had been in storage about 4 months, and the California apples one to 4 weeks at 33°F.(1°C.) prior to use. Storage history of the eastern apples was not available. Variations in maturity were minimized by selection for uniform size and comparable ripeness; flesh color as well as skin color was used to judge ripeness. The fresh texture of different lots investigated separately ranged from hard and succulent to very soft and mealy, but all apples used were sound. In the seasons of 1950 and 1951 the heating tests were continued, using the same varieties except for the eastern apples.

Chemical analyses and heating experiments[b] also were made on apples of the 1947 and 1948 seasons and included the Delicious, Gravenstein, Jonathan, Newtown Pippin, and Winesap. Except for the Gravensteins these were also from Washington State. The California Gravensteins had been stored from 10 days to 7 weeks at 33°F.(1°C.), the others about one month prior to use. The same criteria were used for selection of samples and all apples tested were sound.

Heat treatments. Radially longitudinal wedges were prepared from each variety and heated 20 minutes in steam on a screen platform suspended over boiling water in a beaker capped with a watch glass. About 4 wedges were used for each experiment and these were not placed on the screen until the water in the beaker was boiling so that each sample received essentially the same heat treatment. All wedges were approximately one-half inch in greatest thickness and samples were taken from different sides of each apple. Two or more apples per sample were used to insure reasonably uniform sampling.

Because apples contain large amounts of intercellular gas, some samples were infiltrated while submerged with cold water to learn the effects of gas removal upon cellular changes when subsequently heated. One period of infiltration for 15 minutes was found sufficient for nearly complete gas removal (as judged by the translucency of the wedges) because of the very large intercellular spaces in the fleshy portion of the fruit.

In order to gain further insight into the mechanisms of cell separation and the influence of calcium salts upon the middle lamella materials, raw apple wedges also were subjected to calcium chloride treatments as follows: (a) vacuum infiltration with 0.1 or 1.0% $CaCl_2$ for 15 minutes prior to steaming; (b) vacuum infiltration as in (a), then soaked 30 minutes in the same solution before heating; (c) soaked without infiltration for 30 minutes in $CaCl_2$ solutions before steaming.

Each steam heating experiment, with or without pretreatment, was repeated a number of times on replicate samples with nearly identical results and a corresponding series of samples was heated in boiling water. Observations were made throughout the course of heat treatments. No attempts were made to obtain quantitative measurements of texture in either fresh or heated samples. A simple qualitative appraisal of texture

[b]Appreciation is expressed to Mrs. Doris Taylor of this laboratory, who supplied results of some of these earlier cooking tests and devised the heating test used here.

by pressure with the fingers and the appearance of the heated tissue, after cooling to room temperature, was considered sufficient for histological purposes.

Intracellular change as well as the comparative degree of cell separation resulting from treatments were studied microscopically. Heated tissues were examined by carefully gouging out small samples from the centers of the wedges. Each bit of tissue was mounted in a drop of water on a micro-slide and a cover glass was placed over it. Two or 3 light taps on the cover glass then flattened or partially crushed any cells which did not readily separate. Preparation of several samples in this manner from each experiment provided a ready microscopic comparison of the ease of cell separation obtained with different apples. Fragments of the sauced tissues were merely transferred to the micro-slide with a pipette.

Histological preparations also were made of fresh tissues for each variety. Fresh sections several cell diameters (ca. 500-1,000 μ) in thickness were examined both before and after vacuum infiltration. Some sections were stained and preserved as described elsewhere *(9)*. Similar sections also were subjected to heat treatments on micro-slides to verify observations made on treated wedges. Photomicrographs were taken on a fine-grain panchromatic film.

Analytical. Apples for analyses were peeled, cored, trimmed, and cut into 12 equal wedges. Two pairs of opposite wedges were taken from each apple for analysis. Triplicate samples of 400 g. each, so obtained, were then macerated in an equal weight of 95% ethyl alcohol in a mechanical blendor for 2 minutes. The blendor was sampled by a dipper while turning at low speed.

Total solids were determined by drying the macerated sample to apparent dryness on a steam bath and then for 24 hours at 122°F.(50°C.) *in vacuo*. Total acidity was determined by titration to 7.5 pH. Sugars were estimated by a modification of the ceric sulfate method of Hassid *(5)*. Starch and pectin analyses were made on the dried, alcohol-insoluble solids; starch by the method of Hassid *et al. (6)*, and pectins by the Carré-Haynes method *(2)*.

Because some apples were found to undergo complete cell separation upon vacuum infiltration with cold water, brief comparative analyses were made of removable middle lamella pectins in such an apple and also in one which did not slough with this treatment.[b] This was done with 1949 samples of Cortland and Delicious apples. Two hundred g. of the flesh of every variety were cut into half-inch dice and vacuum infiltered for 30 minutes. Each sample was then filtered and the crude soluble pectins obtained from the filtrates by precipitation with 2 volumes of alcohol. The precipitates and flesh residues were dried *in vacuo* at 140°F.(50°C.). Methoxyl contents of the crude pectins, total solids of the flesh, and the calcium contents of the flesh residues were determined.

MICROSCOPIC OBSERVATIONS

Tissue structure. Figures 1 and 2 show fresh, untreated sections of Newtown Pippin and Rome Beauty, respectively. In Figure 1, which shows parenchyma tissue from about the middle of the apple flesh, dark areas are caused by trapped intercellular gas bubbles which are not in focus; some of the intercellular spaces were open to the cut surface so that gas in these was replaced with water in which the section was mounted. The smaller cells and intercellular spaces of the skin region, as shown in Figure 2 for

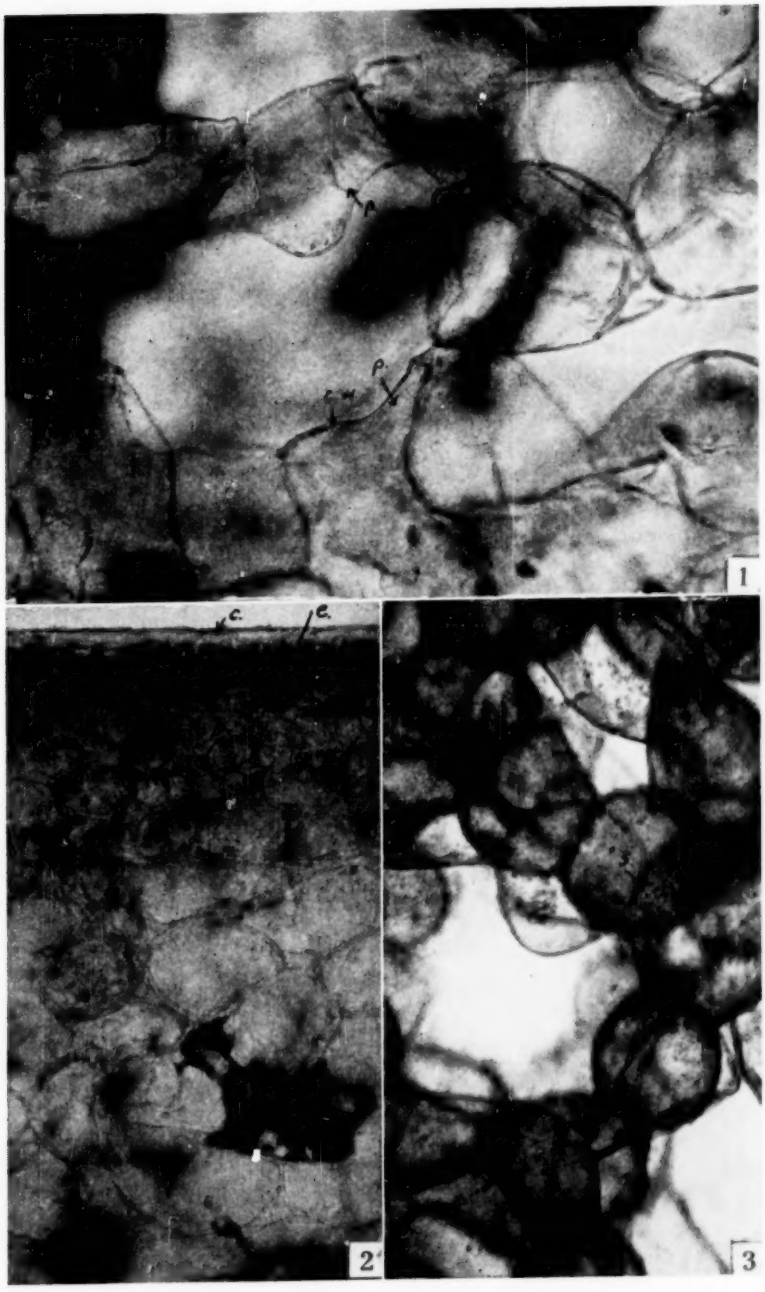
[b]Appreciation is expressed to Dr. R. M. McCready of this laboratory for these analyses.

Figures 1 to 3. Sections of apple flesh showing natural cell structure.

Figure 1. Fresh section Newtown Pippin; p—protoplast, c. w.—cell wall. × 200. Protoplasts in pulp cells consist of very thin film of protoplasm lining cell wall and of minute strands of protoplasm extending through central vacuole which contains cell sap.

Figure 2. Fresh section Rome Beauty showing skin and underlying pulp tissue; c—cuticle, e—epidermis. × 150. Dark irregular bodies in figures 1 and 2 are trapped air bubbles in intercellular spaces.

Figure 3. Histologically prepared thick section of green Gravenstein, dehydrated in alcohol and xylene and mounted in balsam; s—starch grains. × 150.


P
P
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1
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C.
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Rome Beauty, vary in depth for different varieties but seldom extend more than a millimeter from the surface. In Figure 3 a histologically prepared section of the *parenchyma* from the middle flesh region of Gravenstein is shown. The intercellular spaces are filled with the resin mounting medium. The spongy cellular structure of the flesh parenchyma is essentially the same in all varieties and it can be seen in each of these sections that many of the cells are in only partial contact with one another. The middle lamellae by which the cells are coherent lie only between the adjoining cell walls.

Effects of vacuum infiltration and heat on cell structure. Examination of fresh sections before and after vacuum infiltration showed that the cells become distended as as a result of the osmotic uptake of water. Cold water vacuum infiltration of most thick sections of Cortland, Gravenstein, McIntosh, and Rome Beauty, particularly when ripe, resulted in tissue sloughing. The sloughed pulp was composed of intact, separated, and distended cells (Fig. 4). In no instances, however, were ruptured cells found; the cellulosic walls apparently were sufficiently elastic to withstand considerable distension.

Figures 5 and 6 show cells separated by heat treatment of wedges. The cells separate either singly or into small clusters and show no evidence of ruptured walls. The only damage found, as a result of heat treatments used, was the weakening of the middle lamellae and the coagulation of the protoplasmic contents of the cells. The coagulated protoplasts appeared more coarsely granular than in fresh, normal tissues. There was little shrinkage of the coagulated protoplasts in tissues heated without prior infiltration until the tissues stood for 20 or 30 minutes prior to microscopic examination.

Tissues vacuum infiltrated under water and then heated nearly always showed pronounced protoplasmic shrinkage immediately after heat treatment (Fig. 7). This difference may be explained by changes in tissue turgor. The infiltrated tissues were at a maximum turgidity due to osmosis; upon heating, the internal pressures of the vacuum-treated cells changed more drastically than in those heated without prior infiltration. Similar conditions of structure change with heat treatments, and the significance of turgor pressure to physiology and texture have been described for other fruit and vegetable tissues *(12)*.

EFFECTS OF HEATING ON APPLE WEDGES

Progressive changes. The results of the steaming experiments for 1949 apples are summarized in Table 1. Except for the Cortland, McIntosh, and Gravenstein, essentially the same observations were made in heating tests on apples of the 1947, 1948, 1950, and 1951 seasons. The textural behavior thus may be compared with results of chemical analyses for the 1947 and 1948 seasons. Cortland and McIntosh were investigated for only the one season; Gravenstein, however, represents a special situation which is described later.

Apples which tended to sauce in steam (as in Table 1) disintegrated more readily in boiling water because the boiling mechanically aided in loosening the tissue. The general results, however, including the visible effects of heat upon cell structure and cell contents, were essentially the same in boiled and steamed wedges.

Briefly, the phenomena observed with all steamed samples of Gravenstein were (a) a fine effervescence of bubbles of escaping gases when surfaces were first exposed to steam, (b) increase in size of escaping gas bubbles and a rapid development of frothiness within one or 2 minutes, (c) enlargement and merging of intercellular spaces to form large, internal cracks, and (d) the beginning of sloughing in the loci of the cracks. In more extreme cases these stages developed so rapidly that sloughing was simultaneous with frothiness in steamed wedges. Ripe Rome Beauty and Delicious samples were comparable to Gravensteins in these changes when steamed but did not undergo as pronounced sloughing.

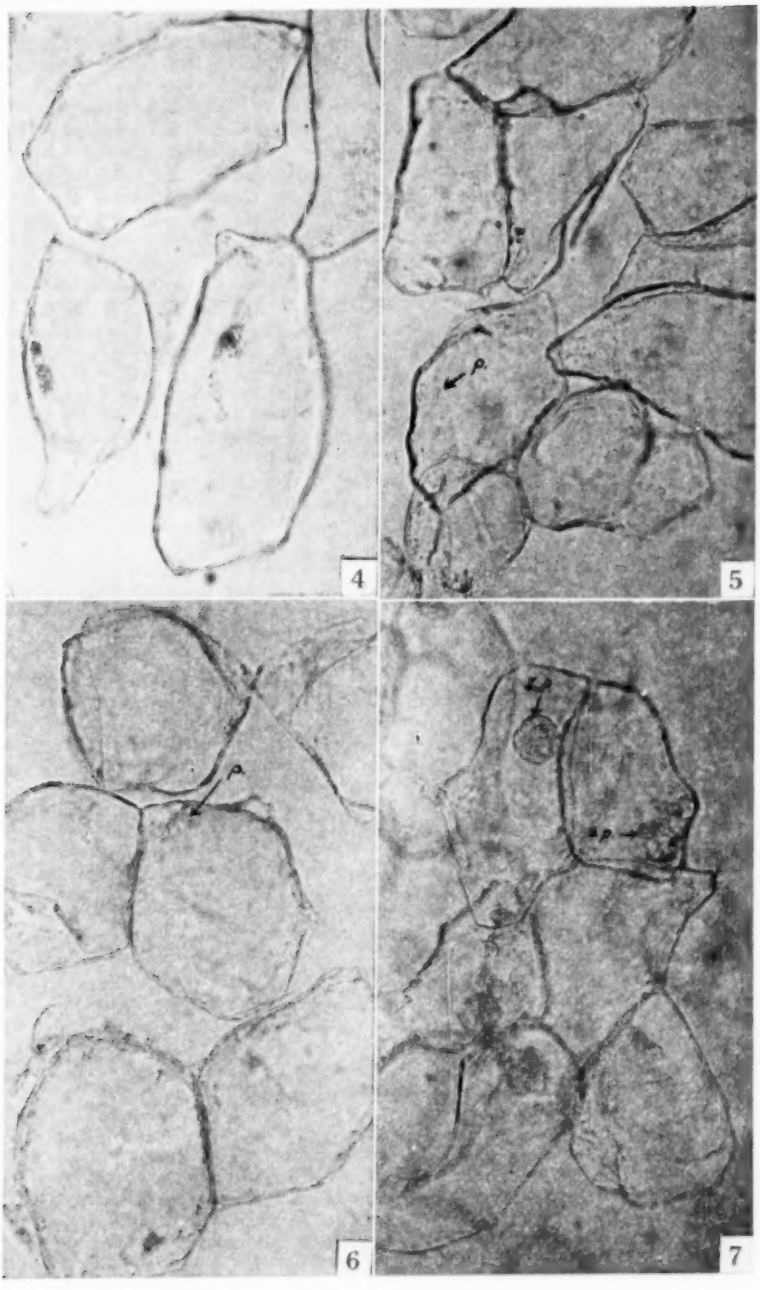
Figures 4 to 7. Separated cells of vacuum-infiltrated and steamed apples. × 150.

Figure 4. Cortland variety, cells separated by vacuum infiltration in cold water.

Figure 5. Rome Beauty, cells separated by steaming; p—coarsely granular, coagulated protoplasm.

Figure 6. Gravenstein, cells separated by steaming.

Figure 7. Cortland, cells separated by vacuum infiltration and then steamed; s. p.—shrunken protoplasts.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6
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TABLE 1

Summary of texture changes in 1949 apples steamed for twenty minutes

Variety, condition and storage history	Texture when cool after steaming only	Effect of vacuum infiltration in water on texture when steamed and cooled	Effect of vacuum infiltration in $CaCl_2$ on texture when steamed and cooled
Winesap, C grade, ripe, firm, succulent. 4 months at 33°F.	Wedges intact, firm, juicy; cell separation not readily obtained on microslide.	Wedges intact, firm, juicy.	Very pronounced firming.
Newtown Pippin (Calif.), ripe, firm. 4 months at 33°F.	Wedges intact, firm, juicy; cell separation not readily obtained.	Wedges intact, firm but slightly mealy.	Very pronounced firming.
Newtown Pippin (Northwest), C grade, ripe and very slightly mealy. 4 months at 33°F.	Wedges intact but mealy; some cell separation obtained.	Wedges intact but soft and mealy; cell separation obtained.	Appreciably firmed; cell separation not readily obtained.
Jonathan, C grade, ripe, firm, succulent. 4 months at 33°F.	Wedges intact but slightly soft; slight cell separation obtained.	Wedges intact, soft; cell separation obtained.	Appreciably firmed; cell separation not readily obtained.
Delicious, C grade, ripe, firm, succulent. 4 months at 33°F.	Wedges intact but slightly soft; cell separation obtained.	Wedges intact but softer; cell separation readily obtained.	Appreciably firmed; cell separation not readily obtained.
Delicious, C grade, ripe, mealy. 4 months at 33°F.	Some sloughing but wedges nearly intact and very soft; cell separation readily obtained.	Surface sloughing with infiltration and more pronounced upon steaming.	Slight surface sloughing with infiltration; wedges firmed and intact after steaming; some cell separation obtained.
Rome Beauty, C grade, ripe, very slightly mealy. 4 months at 33°F.	Some sloughing, wedges only partly intact, very soft.	Tendency to slough when infiltrated; wedges sauced when steamed.	Surface sloughing upon infiltrating; wedges tend to remain intact but very soft when steamed.
McIntosh, Fancy, ripe, mealy. 5 months at 33°F. at this laboratory	Some sloughing in 3 minutes; wedges completely sauced in 10 min. or less.	Virtually complete cell separation when infiltrated.	Virtually complete cell separation when infiltrated.
Cortland (same as McIntosh)	As for McIntosh.	As for McIntosh.	As for McIntosh.
Green Gravenstein (1947 location), succulent and firm. 1 week at 33°F.	Frothed explosively and sloughed completely in 3 to 7 minutes.	Virtually complete cell separation when infiltrated.	Virtually complete cell separation when infiltrated.
Green Gravenstein (1948 location), succulent and firm. 1 week at 33°F.	Slight sloughing but wedges mostly intact although soft; cell separation readily obtained.	Considerable cell separation when infiltrated.	Cell separation usually obtained when infiltrated.
Ripe Gravenstein (1947 location), soft, mealy. 1 week at 33°F.	Wedges sauced in 3 to 7 min.	Virtually complete cell separation when infiltrated.	Virtually complete cell separation when infiltrated.
Ripe Gravenstein (1948 location), soft, mealy. 1 week at 33°F.	Wedges sauced after 15 to 20 minutes.	Virtually complete cell separation when infiltrated.	Virtually complete cell separation when infiltrated.

Differences among apples. All varieties displayed the first three stages of the above changes in steam but showed some variation in amount of frothing. Mealy apples tended to froth less severely than did those less ripe and more succulent in fresh texture. Among varieties which did not sauce with steaming, only Winesap showed very pronounced internal cracking. This variety was singular in this respect; its internal cracking was as severe as that observed in Gravensteins, yet the heated Winesap wedges were intact and somewhat firm when cooled.

Among the firmer apples of comparable ripeness a definite order of decreasing firmness with heating was nearly always observed. Winesaps were usually the firmest and Newtown Pippins from California were nearly as firm when heated. Jonathans and Newtown Pippins from the Northwest were less firm and frequently soft but still intact after heat treatment. Delicious sometimes sloughed but generally remained intact although very soft after steaming.

In earlier cooking tests (1947 and 1948) pronounced differences in rates of saucing were observed with Gravenstein. Those of the 1948 season frothed explosively and often sloughed severely when steamed for only 2 or 3 minutes, irrespectively of maturity. Most Gravensteins tested in the 1947 season sloughed after 10 or 12 minutes of steaming, and saucing was slightly more pronounced with riper than with greener fruit. The 1948 apples had been harvested from a different orchard from those of the 1947 season. Obtainable information on seasonal and edaphic factors, however, showed no correlation with these differences in saucing rates.

In 1949, Gravensteins from both 1947 and 1948 orchard sites were compared. Results tended to negate any possible influence of location upon textural behavior. Differences in rates of saucing were much less pronounced and actually were slightly the reverse of former results. That is, 1949 samples from the 1947 orchard site showed more severe sloughing when steamed than did those from the 1948 location. No reasonable explanations could be found for these differences; the orchard locations were close together so that their climates were identical. It is plausible that physiological differences, such as tissue turgor and gas contents, in the fresh tissues may have been contributing factors to these textural variations.

Effects of vacuum infiltration and of calcium chloride. The influence of vacuum infiltration in water and in 0.1% $CaCl_2$ upon texture obtained with steaming also is listed in Table 1. Generally, all apples which disintegrated when infiltrated with cold water also underwent nearly complete cell separation when infiltrated with either 0.1 or 1.0% $CaCl_2$ so that steam treatment was not practical. Soaking wedges in distilled water tended to increase sloughing rate of all saucing apples when steamed but the increase was not as marked as that produced by infiltration. Soaking in 0.1% $CaCl_2$ had very little firming effect on apples which sloughed rapidly when steamed without pretreatment. Those apples which were firmed by soaking in $CaCl_2$ were either normally firm varieties or the greener of those saucing apples which did not tend to sauce readily when steamed. Essentially the same was true of comparable apples infiltrated with $CaCl_2$ solutions excepting that infiltration tended to increase sloughing of saucing apples when they were steamed regardless of the concentration of $CaCl_2$.

Soaking in $CaCl_2$ solutions did not reduce internal cracking in any varieties when the wedges were steamed. Initial effervescence and gas bubbling from wedges infiltrated in $CaCl_2$ solution was scarcely noticeable but, as the wedges became heated throughout, gas and steam evolving from the tissue rendered the wedges opaque and some internal cracking developed.

The wide range in texture qualities (from Gravensteins which sloughed when only infiltrated with water to Winesaps which remained intact after prolonged steaming) seemed to be accentuated by the $CaCl_2$ treatments. That is, Winesaps were overly firmed by $CaCl_2$ infiltration, while this treatment tended to increase cell separation in Gravensteins. In one experiment not previously described, wedges of ripe and succulent Winesaps were infiltrated with, and then boiled in, 0.1% hydrochloric acid for 30 minutes without reducing tissue sloughing. These wedges were appreciably softer than those subjected to ordinary steaming but the tissues remained intact and only partial cell separation was obtained by pressure on the microslide. These conditions emphasize the importance of the composition of the middle lamellae in the textural behavior of apples.

ANALYTICAL RESULTS

Proximate composition. Table 2 summarizes results of the chemical analyses (total solids, acidity, and carbohydrates) for the varieties investigated in 1947 and 1948. Except for Delicious, a typically low-acid apple, there do not appear to be significant differences in total acidity. Winesap and Newtown Pippin have a slightly greater total solids content than the other varieties. Apparently there are no significant differences in sugars and starch. Some differences in soluble and insoluble pectins between Delicious, Gravenstein, and Newtown Pippin are apparent and there appear to be significant differences between 1947 and 1948 Gravensteins in pectic materials. Relation of these differences to textural qualities will be discussed later.

Water-soluble pectin obtained by vacuum infiltration. A marked difference was found between Cortland and Delicious in their cold water-soluble pectins removed by simple vacuum infiltration. The Delicious sample remained intact but the Cortland underwent complete cell separation when atmospheric pressure was restored after infiltration with water. Thus, in the latter, it is assumed that virtually all of the middle lamella materials were removed or solubilized.

The crude pectins obtained from the filtrates of infiltrated Cortland and Delicious were 0.31 and 0.08%, respectively. Methoxyl content of this Cortland pectin was 9.9%; that of Delicious, 7.6%. Each sample of tissue contained 12.6% total solids and the calcium contents of their dried flesh residues (after filtering) were 0.034 and 0.026%, respectively, for Cortland and Delicious.

Although the distinction between these apples as to cell separation and methoxyl contents of their crude water-soluble pectins suggests that their pectic fractions are considerably different, it cannot be said that the crude pectin so obtained from Delicious represents the soluble pectins of its middle lamellae, because cell separation did not occur. Likewise, relationship of methoxyl contents to textural qualities cannot be assumed because other pectin analyses were not obtained. The difference in calcium contents of the flesh also has no specific meaning. Actually, the results are the reverse of those expected were it inferred that calcium in the flesh reflects amounts present as calcium salts in the pectic materials of the middle lamellae.

Both the ready cell separation and the larger amount of water-soluble pectin obtained with infiltration of the Cortland sample suggest that most of the pectic materials so removed were appreciably degraded. Presumably, the sloughing caused by vacuum infiltration of Gravenstein, McIntosh and some Rome Beauty apples with cold water, as previously described, is a result of a comparable degradation of their middle lamella pectins.

DISCUSSION

Although the foregoing observations demonstrate that water-soluble pectins in the middle lamellae of fresh tissues exert a marked influence upon textural qualities of apples, a brief consideration of other compositional factors is pertinent. Examination of data in Table 2 negates any general correlation of texture with differences in pectins revealed by the Carré-Haynes method. For instance, the average ratio of soluble to insoluble (proto-) pectin is higher in Gravensteins than in any other varieties listed. Green Gravensteins of 1948, however, sauced very rapidly and more severely than did either green or ripe Gravensteins of 1947, yet the latter contained proportionately more soluble pectin.

It also does not seem likely that the slight differences in total acidity in 1947 and 1948 Gravensteins are sufficient, on the basis of hydrolytic action alone, to explain differences in texture when these apples were heated. Correlation of acidity with ease of sloughing when heated is even less satisfactory if other varieties are compared in Tables 1 and 2. The pH of juices of these varieties ranged from 3.5 to 4.1 and this, *a priori*, does not seem a sufficient difference to explain the textural qualities observed. Delicious, intermediate in sloughing tendency, is a low-acid apple; Winesap, the

TABLE 2

Proximate composition of edible portion of apples

Variety and storage time at 33°F. Ripe, except where noted.	Total solids, percent[c]	Total acidity, percent[d]	Reducing sugar, percent[e]	Total sugar, percent[e]	Ratio reducing to total sugar	Total sugar: acid ratio	Starch, percent[f]	Cold-water-soluble pectin, percent[g]	Insoluble pectin, percent[g]
Delicious, C grade (1947), 1 month	15.3	0.19	9.68	12.03	0.81	63.3	0.03	0.11	0.29
Delicious, Fancy (1947), 1 month	14.6	0.19	9.51	11.65	0.82	61.3	0.03	0.09	0.34
Gravenstein (1947), 10 days	14.9	0.45	8.51	11.39	0.75	25.3	none	0.09	0.16
Gravenstein, green (1947), 16 days	14.0	0.51	8.62	10.54	0.82	20.7	0.12	0.20	0.30
Gravenstein, green (1948), 7 weeks	13.7	0.58	8.48	10.24	0.83	17.6	none	0.06	0.35
Jonathan, C grade (1947), 1 month	15.0	0.57	8.54	11.10	0.77	19.3	0.02	0.05	0.31
Jonathan, Fancy (1947), 1 month	15.2	0.57	8.62	11.10	0.78	19.3	none	0.06	0.30
Newtown Pippin (1947), 1 month	17.0	0.44	8.90	13.06	0.68	29.7	none	0.12	0.42
Winesap, Fancy (1947), 1 month	15.8	0.45	9.05	11.64	0.78	25.9	0.04	0.05	0.34
Winesap, Fancy (1948), 1 month	17.7	0.49	9.35	11.55	0.81	23.6	none	0.04	0.35

[c] *In vacuo* at 50°C. [d] By titration to pH 7.5. [e] Hassid (*6*). [f] Hassid, *et al.* (*7*). [g] Carré-Haynes method (*3*). The insoluble ("protopectin") obtained by hydrolysis with 0.33 *N* HCl after removal of cold water soluble fraction; both fractions precipitated as calcium pectate.

firmest of the varieties here, has a high acid content and, as previously mentioned, did not slough when boiled in 0.1% hydrochloric acid. It is not suggested, however, that pH changes in storage may not relate to subsequent changes in texture. Comparisons here are between apples "as received" and do not apply to metabolic changes in storage.

The failure to obtain tissue firming with $CaCl_2$, either in apples which sloughed severely when steamed or in those which underwent cell separation when vacuum infiltrated with cold water, indicates limits to the successful use of $CaCl_2$ for tissue firming. The fact that virtually all of their middle lamellae (and by inference, of the pectic materials) were water-soluble in fresh tissue of such fruit, basically differentiates saucing from non-saucing apples.

Size of cells and intercellular spaces, their intercellular gas contents and physiological conditions of cell turgor in apples may contribute to texture changes, as previously mentioned. Escape of expanding intercellular gases during heating appears to have an appreciable mechanical influence upon tissue sloughing in some apples, such as Gravenstein. In others, such as Newtown Pippin and Winesap, the middle lamellae are sufficiently resistent that sloughing during heating does not readily occur in spite of severe frothing and internal cracking.

Because of various histological conditions and the wide range of textural qualities which different apples exhibit, instruments for measuring firmness of fruit at harvest maturity may not provide information pertinent to particular texture problems with processed apples. Such instruments register the sum-total mechanical resistance of structure, such as toughness of cell walls, tissue turgidity, and relative porosity; thus the influence of middle lamella materials may be masked. For predicting texture qualities in processed apples, simple heating tests probably have a more practical value.

SUMMARY

Observable differences in textural qualities of several western and 2 eastern varieties of apples subjected to experimental heating showed no consistent relationships to factors of proximate composition. Saucing or tissue sloughing of Gravensteins when steamed varied in different seasons but this variation showed no significant correlation with ratio of soluble to insoluble (proto-) pectins as revealed by the Carré-Haynes method. Tissue sloughing, due to separation of intact cells, was aided by expansion and escape of intercellular gases during heat treatments. All apples showed considerable internal cracking during steaming as a result of escaping gases; this internal cracking did not cause tissue sloughing in firm, non-saucing apples (Winesap, Newtown Pippin, Jonathan, and greener Delicious). With saucing apples (riper Delicious, Gravenstein, Cortland, McIntosh, and Rome Beauty) the development of internal cracking during heating initiated tissue sloughing.

Most saucing apples underwent complete cell separation when vacuum infiltrated with either cold water or calcium chloride solution. Soaking such apples in calcium chloride solution prior to steaming produced no firming effect upon the tissue. Calcium chloride treatments of non-saucing apples

increased firmness after heating; these apples did not undergo cell separation when vacuum infiltrated with water prior to heat treatment.

When cell separation resulted from vacuum infiltration it appeared that nearly all middle lamellae materials had been removed or solubilized. The cold-water-soluble pectic fraction so removed from a rapidly saucing apple, Cortland, was 4 times (0.31%) that obtained from Delicious (0.08%), an apple of intermediate textural qualities which did not slough severely upon infiltration and which could be firmed with calcium chloride.

These observations and analytical results indicate the inadequacies of conventional methods of analyses for elucidating mechanisms of texture change in apples. It is concluded that more fundamental investigation of all factors of composition is needed for a better understanding of differences in fresh texture between varieties, or even within the same variety. The chemical nature and solubility properties of pectins and other middle lamellae materials, as they occur *in situ* and as they are affected by processing, require further clarification. In addition, more knowledge of the cellular structure of apple flesh and of distribution of its intercellular spaces, in different varieties and as affected by processing, would be pertinent.

In the absence of more exact information it is suggested that simple cooking tests, such as steaming in combination with vacuum infiltration of tissues, have practical value in testing apples for their textural qualities.

LITERATURE CITED

1. Branfoot (Carré), M. H. A critical and historical study of the pectic substances of plants. Dept. Sci. and Ind. Res. (British), *Food Inv. Sp. Report 33* (1920).
2. Carré, H. H., and Haynes, D. The estimation of pectin as calcium pectate and the application of this method to the determination of soluble pectin in apples. *Biochem. J.*, **16**, 60-69 (1922).
3. Esau, K. *Plant Anatomy*. 1953, John Wiley and Sons, Inc., New York.
4. Griffin, Jean H., and Kertesz, Z. I. Changes which occur in apple tissue upon treatment with various reagents and their relation to the natural mechanism of softening during maturation. *Bot. Gaz.*, **108**, 279-285 (1946).
5. Hassid, W. Z. Determination of reducing sugars and sucrose in plant materials. *Ind. Eng. Chem., Anal. Ed.*, **8**, 138-140 (1936).
6. Hassid, W. Z., McCready, R. M., and Rosenfels, R. S. Determination of starch in plants. *Ind. Eng. Chem., Anal. Ed.*, **12**, 138-140 (1940).
7. Kertesz, Z. I. The Pectic Substances. 1951, Interscience Pub., New York.
8. Reeve, R. M. Relation of histological characteristics to texture in seed coats of peas. *Food Research*, **12**, 10-23 (1947).
9. Reeve, R. M. Rapid preparation of thick sections of fleshy plant tissues. *Stain Technol.*, **27**, 29-35 (1952).
10. Scott, F. M. Internal suberization of tissues. *Bot. Gaz.*, **111**, 378-394 (1950).
11. Tetley, U. The morphology and cytology of the apple fruit, with special reference to the Bramley's seedling variety. *J. Pom. and Hort. Sci.*, **9**, 278-297 (1931).
12. Weier, T. E., and Stocking, C. R. Histological changes induced in fruits and vegetables by processing. *Recent Advances in Food Research*, II (edited by E. M. Mrak and G. F. Stewart). 1949, Academic Press, Inc., New York.

HISTOLOGICAL INVESTIGATIONS OF TEXTURE IN APPLES. II. STRUCTURE AND INTERCELLULAR SPACES

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Separation of intact cells as a feature of tissue sloughing in saucing apples was described in a previous paper in which the qualities of several varieties were compared *(16)*. It was observed that expansion and escape of intercellular gases during heat treatments aided mechanically in cell separation of so-called "saucing" apples. Because escape of expanding gas did not cause tissue sloughing in nonsaucing apples, and because frothing or bubbling of gases from these appeared to develop more slowly than from saucing apples, when steamed, it seemed worth while to investigate size of cells and intercellular spaces. Estimations of average cell size, cell-wall surface per unit volume, and of distribution and volume of intercellular spaces in different tissue regions were made for several commercial varieties of western apples. In the present communication the resulting data on structure are evaluated with respect to the importance of middle-lamella pectins to fruit texture.

METHODS

Microscopic. Both fresh and histologically prepared sections from different tissue regions were used for microscopic studies. A few prepared sections were cut from paraffin-embedded tissues. Most of the sections used, however, were cut from fresh apples at thicknesses of 300 to 500 μ on a sliding microtome. These latter sections were then prepared according to methods described elsewhere *(15)*. The final preparations of these thick sections preserved clearly defined intercellular spaces, intact cells and cell contents in their natural spatial relationships. Such sections were ideal for measuring sizes of cells and intercellular spaces and for study under a stereoscopic microscope.

Cell measurements were made with a calibrated eye-piece micrometer at 60 × magnification. Only parenchyma cells considered representative of a given region were measured; for example, those bordering vascular tissues were not included because of their smaller size. All measurements were made with sections from apples of nearly the same size, as later described. From 50 to 100 cells in 4 or 5 sections from each of 3 to 6 apples per variety were measured. In statistical treatment *(22)*, cell dimensions were expressed in terms of eye-piece micrometer divisions. One scale division was used as the interval between classes and the number of cells in each class was recorded. The averages and standard deviations were then converted to microns according to the calibration of the micrometer.

A similar method of sampling was used in measuring intercellular spaces but only radial sections from the equatorial region of the fruit were used. Minute interconnecting channels and tiny spaces between the angles of several abutting cells were not measured because such spaces are mainly in the more compact tissue regions and do not contribute to the open structure of the flesh parenchyma, as shown in Figures 3-7. Only 50 larger spaces, ranging from about 100 to over 1,000 μ in length, were measured for each variety. The smaller size of sample was used for several reasons: Even

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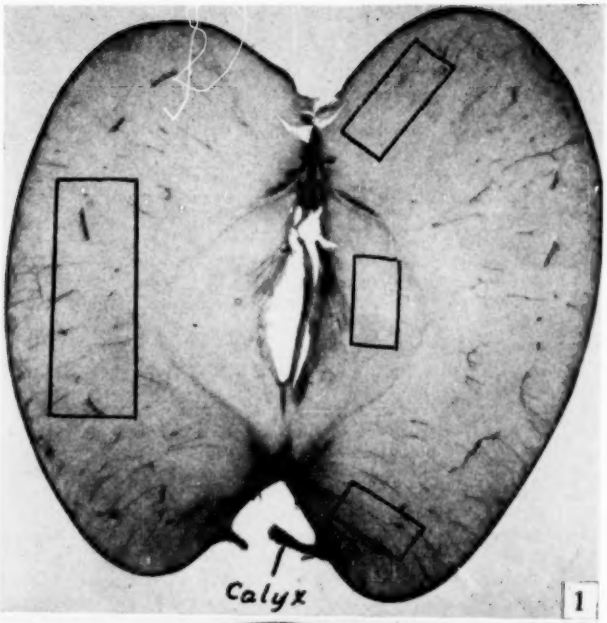
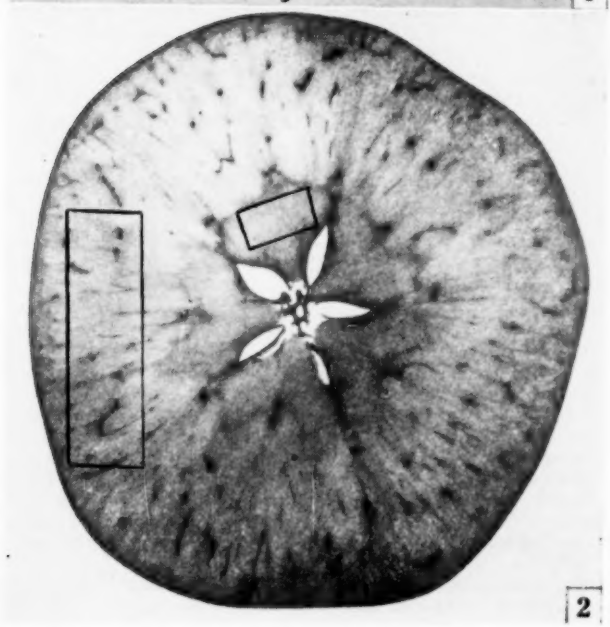




Figures 1 and 2. Transverse and longitudinal entire sections, respectively, of Delicious variety × 1.5. These were prepared similarly to the thick sections shown in some of the other figures but were cut at 1 mm. Areas from which samples were taken for estimations of intercellular space volume are outlined.

in thick sections not all spaces observable are complete, so that many more sections would be required to measure several hundred intact spaces per variety. Likewise, many spaces have lengths exceeding that of the micrometer scale, so that measurements are more tedious than is the case with parenchyma cells. In order to test the reliability of the data obtained with the comparatively smaller sample, a standard error of the standard deviation was used *(22)*. This standard error, a test of the sample against the universe, was found to have a consistent value of only about 10% of the value obtained for standard deviation in each variety.

Estimations of average number of cells, volume of average cell, and cell wall surface per unit volume of tissue also were made. These estimations were based on the assumption that the cells are nearly spherical—that is, that they approach a 14-sided shape which is more or less characteristic of parenchyma tissue in many plants *(3, 8, 9)*. Volume (V_1) of the average cell was obtained from its diameter (D) by $\frac{1}{6}$ πD^3; its cell wall surface (A_1), by πD^2. The volume of tissue actually occupied by cells (V_2) was obtained as a percentage of total tissue volume when percentage of intercellular space was known. Then, $V_2/V_1 \times A_1 =$ total area of cell wall surface per unit volume of tissue.

In defining V_2 it is necessarily inferred that the spheres of cells packed into V_2 have no intercellular spaces. This is precisely the condition obtaining if the cells were of the proper 14-sided structure and perfectly packed. Models approaching this ideal have been constructed with foam bubbles or by compressing soft lead shot in a suitable container *(11, 12)*.

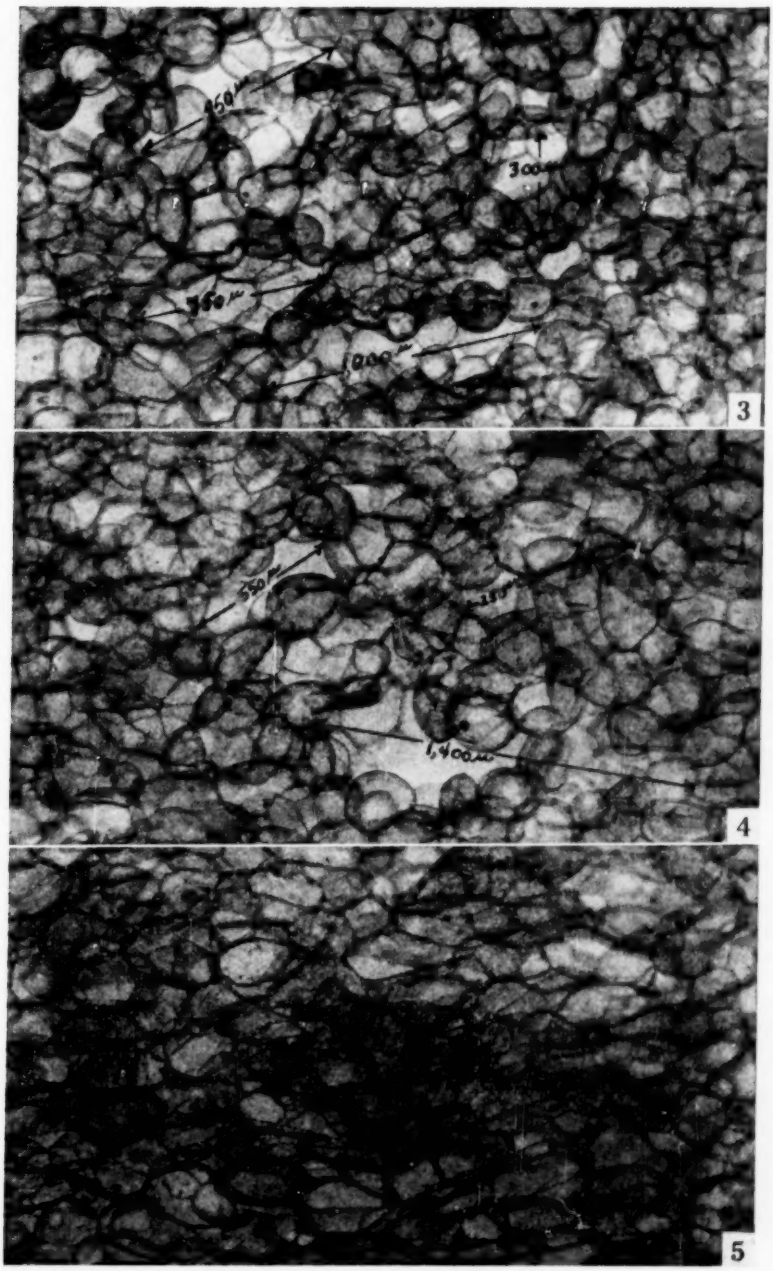
Volumetric. Estimations of the total intercellular space per unit volume of tissue were made by vacuum infiltration of the tissue submerged in either water or sucrose solutions. Both water and sucrose solutions were used in order to learn whether the influence of leaching loss and the effects of greatly increased cell turgor, such as occurs in water, altered the values obtained.

Samples for infiltration were removed from the different tissue zones shown in Figures 1 and 2; these varied in size and shape according to the zone sampled. All samples ranged from 5 to about 20 g. in weight. Sampling of the fleshy middle region of the fruit was accomplished by removing longitudinal strips about $\frac{1}{2} \times 1 \times 3$ cm. Two or more strips were used per sample and each sample was composited from 2 or more apples. About 8 to 10 sets of samples were used in determining intercellular space volume for each variety.

Vacuum infiltration and volume determinations were accomplished as follows: The samples were first weighed in air within a closed screen basket, then weighed submerged in the infiltration medium. Submerged weight also was determined after vacuum infiltration. The original tissue volume was derived from the difference between weight in air and submerged weight prior to infiltration. Change in submerged weight after infiltration was taken as an expression of the volume of the intercellular space in the apple. No significant differences in values obtained for intercellular volume were found between samples that were vacuum infiltrated for 15 minutes or longer. Samples infiltrated less than 15 minutes gave results which were more variable but which averaged about the same as those from replicate samples infiltrated for longer periods.

About a 15-minute interval after restoration of atmospheric pressure was required for most samples to show apparently complete translucency. During this time, uptake of water by osmosis caused the cells to become very turgid so that minute pockets of gas were "squeezed out" of the tissue, and fluctuations in submerged weight could be observed. Submerged weights recorded after this 15-minute interval showed no significant change; consequently, 15 minutes was chosen as a standard time before submerged weight after infiltration was determined. Further interrelationships of osmosis, turgor, and cell volume are discussed by Meyer and Anderson *(13)* and briefly by Weier and Stocking *(23)*.

Effects of leaching loss upon submerged weight after water infiltration were considered to be negligible. Although up to 19% of the total sugars may be leached when apple slices are soaked for 30 minutes in water *(14)*, the actual weight of solutes lost is comparatively small. It can be calculated that weight of solutes lost from water-infiltrated tissues does not cause significant change in the submerged weight of the tissue. Replicate samples vacuum-infiltrated with water and with different sucrose solutions showed nearly identical values for intercellular volume.



Figures 3 and 4. Transverse radial sections of flesh of apples about 1/8" beneath skin showing lengths of some larger intercellular spaces, × 40. Fig. 3—Newtown Pippin; Fig. 4—Gravenstein.

Fig. 5. Transverse radial section of core parenchyma of Newtown Pippin, × 40.

MATERIALS

Because some of the apples used for the present observations were of different sources from those used for previously described heat treatments *(16)*, possible influences of sampling upon results were carefully checked. Comparisons made between apples of similar size and maturity, but of different sources, within each variety obtained from the Pacific Northwest showed no significant variations in size of cells and amount of intercellular space.

Of necessity, selection of fruit in each variety was entirely subjective. Apples appearing to be characteristically of average size for each variety were used, excepting apples deliberately selected for size variation. Fruit diameter was not used as a criterion of size because of varietal differences in fruit shape. Selection for comparable maturity in each variety was based upon such characteristics as general texture of the flesh and flesh and skin color. Thus, except where noted later, the results may be considered to represent sound apples of similar size and maturity which had been in storage one to 3 months at 33°F.(1°C.). These apples were allowed to stand a day at room temperature before sampling. Holding the fruit 3 to 4 days had little or no effect upon results except for ripe Gravenstein and Rome Beauty, which sometimes became too mealy for successful vacuum infiltration.

Estimations of intercellular spaces in some ripe Gravenstein and Rome Beauty apples required slight modification of the method. Frequently, these apples underwent complete cell separation when vacuum infiltrated. When firmer ripe apples were selected and infiltrated in 1% calcium chloride solutions, cell separation sometimes was prevented so that intercellular volume could be measured.

RESULTS

Microscopic observations. Except for slight variations in the distribution of vascular tissues, comparable tissue regions appear to be similar in all varieties investigated. The different regions, however, are distinct from one another in size and shape of parenchyma cells and of intercellular spaces. Table 1 summarizes some of these characteristics.

TABLE 1

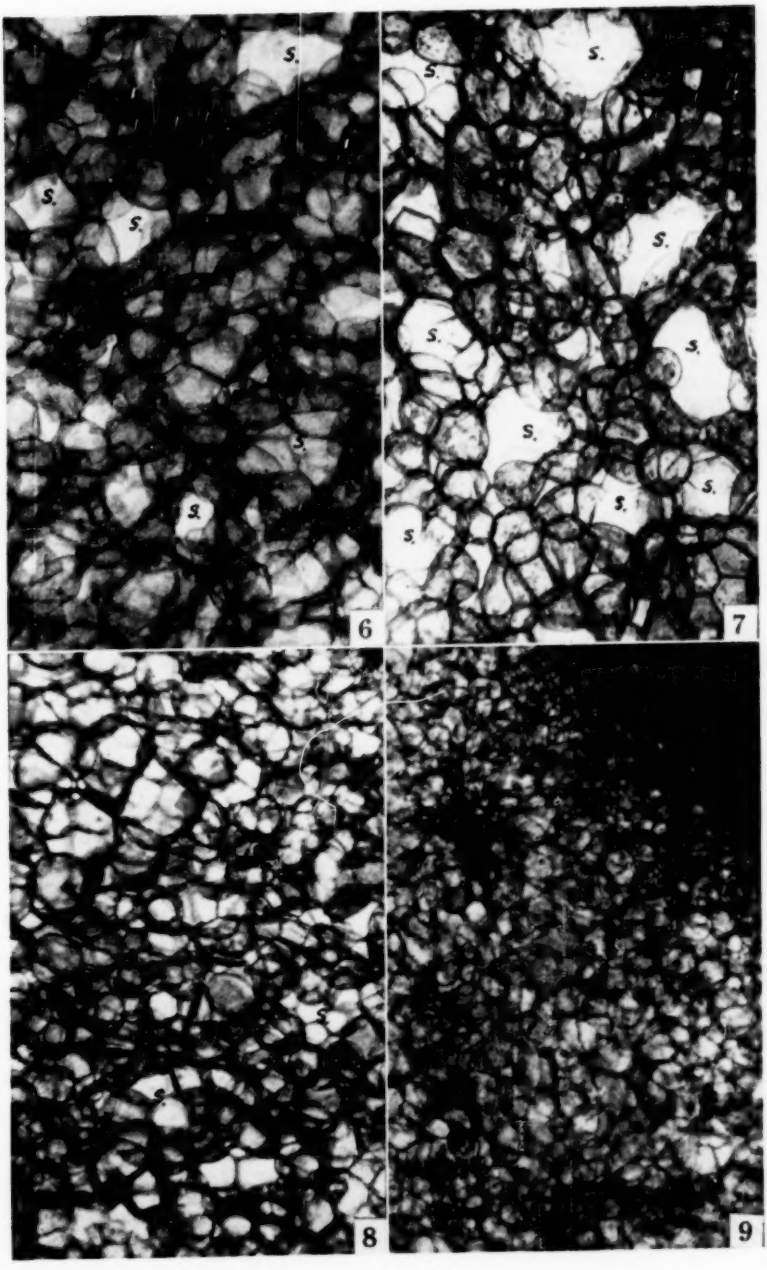
Dimensions (in microns) of parenchyma cells in different tissue regions of apples

Variety	Skin		Outer flesh $\frac{1}{8}$"-$\frac{1}{4}$" depth		Inner flesh $\frac{3}{8}$"-$\frac{5}{8}$" depth		Core (length)		Core (width)	
	Av.	SD[b]	Av.	SD	Av.	SD	Av.	SD	Av.	SD
Newtown Pippin	76	± 16	182	± 42	210	± 39	261	± 58	136	± 29
Winesap	67	±21	194	± 42	214	± 35	266	± 42	142	± 33
Delicious	67	± 18	161	± 27	170	± 30	266	± 57	124	± 25
Rome Beauty	67	± 24	193	± 34	207	± 29	327	± 72	142	± 21
Gravenstein	85	± 27	221	± 43	235	± 58	494	± 95	157	± 32

[b] SD = Standard Deviation, or $i\sqrt{\left(\frac{\Sigma(fd^2)}{n} - \frac{\Sigma(fd)^2}{n}\right)}$, where i = class interval, f = class frequency, and d = deviation from av.

In all varieties, smaller cells and intercellular spaces occur in the calyx end than in the equatorial regions of the fruit. Likewise, the stem end near the base of the core contains smaller cells and intercellular spaces than in most of the fleshy portion. Parenchyma cells closely associated with vascular bundles and with the hard layer of the seed cavities also are smaller and somewhat elongated in comparison with surrounding cells.

There are no marked differences between Newtown Pippin, Rome Beauty, and Winesap as to size of flesh parenchyma cells. Delicious and Gravenstein have smaller and larger flesh cells, respectively, than do the other 3 varieties. Somewhat larger core parenchyma cells distinguish Gravenstein and Rome Beauty from the others. A limited number of measurements made of flesh cells in Cortland and McIntosh apples (not listed in Table 1) suggest that these Eastern apples are similar to the Gravenstein in cell size.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Figures 3 to 5 show the core and flesh tissue of the Gravenstein and Newtown Pippin in radial sections. In the equatorial region of all varieties, the cells in the portion towards the skin are slightly smaller than those more deeply located. Many flesh parenchyma cells are slightly elongated but this elongation does not appear to have any definite orientation with respect to the fruit axis. Parenchyma cells of the core, particularly between seed cavities, are characteristically elongated and show a more distinct radial elongation in some varieties than in others.

Nearly all the larger intercellular spaces are radially elongated and have small, interconnecting channels (Figures 3 and 4). Figures 6 to 8 show these large spaces in tangential sections of different thickness in the flesh parenchyma. The smaller cells and intercellular spaces of the skin tissues are shown in tangential section in Figure 9, and in radial section in Figure 10. Many of the cells and spaces in the skin area are slightly flattened radially and thus appear to be elongated in radial sections of the fruit. In tangential sections the skin cells are more uniformly enlarged in directions paralleling the fruit surface.

The considerable range in dimension of the larger intercellular spaces in all varieties is shown by the deviations from averages (Table 2). In length, these spaces average somewhat more than twice the diameters of the surrounding parenchyma cells. In overall length of intercellular spaces the varieties fall into 2 groups: Newtown Pippin, Winesap, and Delicious, which have shorter average spaces than Gravenstein, Rome Beauty, Cortland, and McIntosh.

Intercellular space volume. Some indication of differences in intercellular space in various tissue regions may be gained from Table 3, which compares results obtained by vacuum infiltration. These differences are related to other variations in tissue struc-

Figures 6-8. Tangential sections of flesh of apples about 3/8" beneath skin, × 40. Fig. 6—Gravenstein, section 500 microns thick; Fig. 7—Newtown Pippin (green), section about 200 microns thick; Fig. 8—Winesap, section about 400 microns thick. S—intercellular spaces.

Figures 9 and 10. Sections showing skin area of Gravenstein and Delicious, respectively. Fig. 9—from a tangential section, cells in upper corner include epidermis, × 40; Fig. 10—radially transverse section cut at 15 microns from paraffin-embedded tissue and showing transition from skin to flesh parenchyma; c—cuticle, e—epidermis, s—intercellular space, × 150.

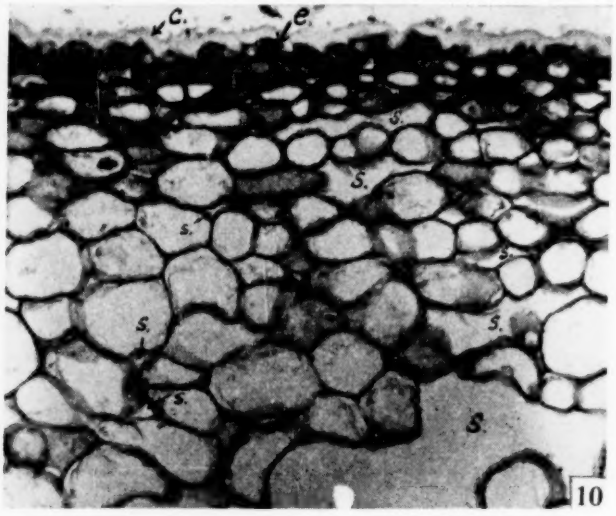

TABLE 2

Dimensions (in microns) of intercellular spaces of middle flesh region of different apples

Variety	Width			Length			Greatest lengths observed
	Av.	SD	SE[c]	Av.	SD	SE[c]	
Delicious	269 ±	93 ±	9	487 ±	154 ±	15	1,000
Newtown Pippin	257 ±	72 ±	7	485 ±	192 ±	19	1,200
Newtown Pippin[d]	259 ±	63 ±	6	438 ±	187 ±	19	1,000
Winesap	275 ±	74 ±	7	480 ±	157 ±	16	1,200
Green Gravenstein	210 ±	99 ±	10	544 ±	210 ±	21	1,300
Ripe Gravenstein	350 ±	108 ±	11	665 ±	274 ±	27	1,500 and 2,100[e]
Rome Beauty	257 ±	75 ±	8	562 ±	231 ±	23	1,800[e]
Cortland	295 ±	74 ±	7	620 ±	233 ±	23	1,600 and 2,000[e]
McIntosh	300 ±	75 ±	7	590 ±	250 ±	25	2,000[e]

[c] SE = Standard error of the standard deviation, or $\frac{SD}{\sqrt{2n}}$ (a test of reliability, Waugh 22).

[d] From Watsonville, California; other Newtown Pippin from Pacific Northwest.

[e] Observed in fresh sections, other data from prepared thick sections.

TABLE 3

Amount of intercellular space in different tissue regions of apples, as determined by vacuum infiltration with water and sucrose solutions. (Where a range is given, number of determinations is in parentheses)

Variety and character of fruit	Core parenchyma between seed cavities	Percent of tissue occupied by intercellular space as determined by vacuum infiltration with water		
		Calyx end flesh	Stem end flesh	Middle portion of flesh
Delicious, ripe, not mealy (1950)	.....	15	18.0	21.0-22.0(3)
Gravenstein,[f] ripe, 1950	9.1	17.6	20.1	25.4-27.0(3)
Newtown Pippin, ripe, 1951	7.2-7.9(3)	13.8-14.2(3)	16.5	21.5-23.6(3)
Rome Beauty,[f] ripe, 1950	6.8-9(4)	13.8	17.9	22.0-23.6(4)
Winesap No. 80,[g] ripe, 1951	5.9-9(3)	.....	.....	22.3-22.5(3)
Winesap No. 113,[g] ripe, 1951	9.5	15.3	17.7	19.8-20.7(3)
Winesap No. 138,[g] ripe, 1951	9.7	13.9	18.6	20.1-20.9(3)

	Percent of middle flesh tissue occupied by intercellular spaces as determined by vacuum infiltration in different concentrations of sucrose solution			
	Water	6% sucrose	8% sucrose	12% sucrose
Gravenstein, green, 1950	25	24.7-24.9(3)	.....	24.5
Gravenstein[f]	25.2-26.3(3)	.....	25.4-26.6(3)	25.7-26.7(3)
Newton Pippin, green, 1950	20.3-21.7(9)	20.5-21.0(3)	19.0-20.1(3)	20.7
Newton Pippin, culls, 1950	17.3-18.5(2)	17.7-19.4(4)	19.1-19.5(3)	16.8-18.3(3)
Rome Beauty,[f] ripe, 1950	(see above)	21.4-23.6(4)	22.0-22.2(3)	22.7-24.7(4)

[f] Vacuum infiltration medium made up with 1% calcium chloride to reduce cell separation.

[g] Size number here refers to number of apples packed in a 40-lb. (cu. bushel) box as per commercial practice in the Pacific Northwest. Diameters vary according to shape of different apples. No. 80's here ranged between 3 and 3½ inches diameter; No. 113's, about 2½ to 2¾ inches; No. 138's, about 2 to 2¼ inches (without regard to U. S. grades).

ture. For example, the core parenchyma comprises one of the most compact tissue regions and has the least amount of intercellular space.

The slight influence of size of the fruit upon intercellular space in the flesh parenchyma is illustrated with 3 sizes of Winesap and 2 sizes of Newtown Pippin. In each instance the larger fruit has slightly more intercellular space than the smaller, but little, if any, difference is shown between the 2 smaller sizes of Winesap apples. The Newtown Pippin culls used here were appreciably undersized or poorly developed. In some culls only about 12 to 14% of the tissue was occupied by intercellular space. Among normally sized apples of each variety, the Gravenstein showed consistently the most intercellular space; next was Rome Beauty with slightly more, on the average, than Newtown Pippin, Winesap, and Delicious.

Influence of fruit shape upon cell size and intercellular space was not investigated within any variety here. Presumably, the less developed side of a lopsided fruit would contain smaller cells and less intercellular space than the fully developed side. Within normal fruit, the differences between varieties in Table 3 for intercellular space of the middle portion of the flesh may reflect the characteristic differences of fruit shape for these varieties. For instance, both Gravenstein and Rome Beauty are somewhat flat and well-rounded, in comparison with Delicious and Winesap in which radial enlargement is proportionately less pronounced.

TABLE 4

Size and number of cells and cell wall surface per unit volume of apple flesh parenchyma

Variety and tissue	Average cell diameter (microns)	Av. wall area per cell ($microns^2$) A_1	Average cell volume ($microns^3$) V_1	Approx. percent occupied by cells V_2[b]	Cells per unit volume (1 cc.)	Total surface of cell wall area per unit volume (1 cc.) A_2	
						A_2 as $microns^2$	As $cm.^2$
Newtown Pippin				%			
Outer flesh[1]	182	1.04×10^5	3.16×10^6	80	2.53×10^5	2.64×10^{10}	263
Inner flesh[1]	210	1.39×10^5	4.85×10^6	80	1.65×10^5	2.3×10^{10}	230
Delicious							
Outer flesh	161	0.814×10^5	2.19×10^6	80	3.66×10^5	2.99×10^{10}	299
Inner flesh	170	0.91×10^5	2.57×10^6	80	3.11×10^5	2.83×10^{10}	283
Gravenstein							
Outer flesh	221	1.53×10^5	5.65×10^6	75	1.32×10^5	2.03×10^{10}	203
Inner flesh	235	1.73×10^5	6.8×10^6	75	1.1×10^5	1.9×10^{10}	190
Rome Beauty							
Outer flesh	193	1.17×10^5	3.71×10^6	78	2.11×10^5	2.47×10^{10}	247
Inner flesh	207	1.35×10^5	4.6×10^6	78	1.69×10^5	2.29×10^{10}	229
Winesap							
Outer flesh	194	1.19×10^5	3.8×10^6	80	2.10×10^5	2.50×10^{10}	224
Inner flesh	214	1.44×10^5	5.13×10^6	80	1.56×10^5	2.24×10^{10}	224

[b] Only approximate percent of unit volume occupied by cells; outer flesh slightly lower and inner flesh slightly higher in intercellular space content. 1×10^{12} = cubic microns per cc. of tissue.
[1] At about $\frac{1}{4}$ inch beneath skin tissue.
[1] At about $\frac{1}{2}$ inch beneath skin tissue.

Total cell-wall surface per unit volume. Table 4 provides further statistical comparison of the flesh structure in 5 of the varieties investigated. The approximate percentage of tissue occupied by cells is derived here from average percentages of tissue occupied by intercellular space (Table 3).

The Gravenstein possesses cells of larger average volume, fewer cells per unit volume of flesh, and less cell-wall surface per unit volume of flesh than do any of the others. Rome Beauty is less different from the Newtown Pippin and Delicious than is Gravenstein in these characteristics. Although showing slight difference in amount of tissue occupied by cells, Winesap, Newtown Pippin, and Rome Beauty appear to be almost identical in structure irrespectively of differences in shape.

In general, the differences between varieties (Table 4) in cell-wall surface per unit volume probably do not reflect as great a difference as may exist between varieties in amount of abutting cell-wall surface. Because of the large amount of cell-wall surface bordering intercellular spaces, an appreciably lower amount of cell wall surface than that listed is in contact with the middle lamellae materials between abutting cells (Figures 3, 4, 6, 7).

DISCUSSION

Relation of structure to processing and saucing. The open structure of apple tissue has a practical relationship to certain processing treatments. Size and orientation of intercellular spaces permit ready impregnation with heavy sirups to enhance the quality of some products *(4)*. Fruits with less open structure are not so readily impregnated. Likewise, some processing techniques may be influenced by the form in which the fruit is prepared, whether in radial wedge, dice, or transverse slice, because of the radial orientation of the larger intercellular spaces. Gas composition in the proportionately large volume of intercellular space also may affect the development of new processing methods such as dehydrocanning and dehydrofreezing.

There is no consistent correlation between structural features of cell size, intercellular spaces, and cell-wall surface and the ease of cell separation or saucing qualities previously described for these varieties *(16)*. But when certain varieties are compared, some relationship to saucing characteristics is apparent. The flesh of the Gravenstein, the most rapidly saucing variety here, has more intercellular space, larger cells, and less cell-wall surface than either Newtown Pippin or Winesap, both of which resist saucing when steamed. The Delicious, on the other hand, is intermediate in saucing qualities and has smaller cells and more cell-wall surface per unit volume of flesh than the other varieties. With Rome Beauty, another saucing apple, cell size and cell-wall surface are similar to those of both Newtown Pippin and Winesap and there is slightly more intercellular space than in these nonsaucing varieties. These inconsistent relationships of structure to cell separation, or saucing, serve to emphasize the importance of middle-lamellae composition to texture in apples.

Different rates of saucing appear to be better related to variations in growth and season of ripening in different apples than to structural features of cell size, intercellular spaces, and cell-wall surface. The Gravenstein is a rapidly growing summer apple while the Newtown Pippin and Winesap are slower-growing fall apples. Distinctions between these two sorts of fruit, as to cell size, etc., reflect natural physiological differences in the metabolism of their growth and ripening which, of course, are of genetic origin. Through such differences in metabolism, variations in amounts of cell-wall surface and in composition of the middle lamellae arise and contribute to the varied textural qualities that apples exhibit.

Relation of structure to growth and storage. The structure of apples has received some attention in investigations on growth, ripening, and storage of fresh fruit. Relatively few histological investigations, however, other than those pertaining to interpretations of fruit morphology and evolution, have been published.

Krause *(7)* reported variations in distribution of vascular tissues between varieties, but noted no differences in flesh parenchyma. Tuckey and

Young *(21)* found that flesh parenchyma cells do not increase in size after the fruit has reached harvest maturity, but that very slow cell enlargement occurs in certain areas, such as the skin, until full ripeness is reached. Tetley *(20)* found that by the time maximum average cell size has been reached, storage starch decreases rapidly—being used during ripening after growth has ceased. These findings are in general agreement with the limited information on maturity and size of fruit in the present report. For example, the less developed fruits (culls) have appreciably less intercellular space and smaller cells than fully developed apples. It seems reasonable that late increases in fruit size, as during ripening on the tree, are most likely due to enlargement of intercellular spaces concomitant with slight cell enlargement in the skin area. These features of growth, however, have not been investigated in detail in many varieties.

In other investigations Smith *(17)* reported that British apples with a short growing season have more cells per unit weight, higher respiration rates, and poorer keeping quality than do those with a characteristically longer growing season. These interpretations are difficult to reconcile with comparable information from the present studies. For example, comparison of Gravenstein with Newtown Pippin and Winesap indicates that smaller and more cells per unit volume of tissue are associated with better keeping quality and longer growing season. When Delicious and Winesap, or Delicious and Newtown Pippin, are compared, however, it appears that larger and fewer cells per unit volume of tissue are correlated with firmness and better keeping quality. It seems obvious that other factors in fruit composition and physiology need be considered for an understanding of keeping qualities. Further, an insufficient number of varieties have been investigated to permit a general appraisal of maturity and storage qualities on the basis of structural features.

More recently Smith *(19)* made an interesting statistical analysis for several varieties of British apples as to changes in cell volume and number of cells per weight and volume of whole fruit from flower to ripeness. Because the statistical treatment was confined to flesh parenchyma cells, it necessarily neglects the influence of differentiation of tissue in the early stages of fruit formation. In these earlier stages the rates of cell formation may be considerably influenced by cell divisions in procambium (or precursory vascular tissues), which may continue at a comparably greater rate than in cells destined to form flesh parenchyma. Also, in the earlier stages of fruit formation, the proportion of precursory vascular tissue to young flesh parenchyma is higher than its relation to volume of flesh parenchyma in the mature fruit. The tissues present in the mature fruit are differentiated at varying rates during fruit development and the characteristics of the cells change markedly as histogenesis proceeds *(21)*. Considerably more histological investigation along the lines begun by Smith *(19)* is needed to clarify the relation of cell processes with over-all fruit growth.

Smith *(17, 18)* also reported that the parenchyma cells of the core in some apples are only about half the size of those in the flesh. His observations were based upon appearance of these cells in sections from paraffin-embedded samples which were cut apparently in a plane per-

pendicular to the radii of the fruit. Because core parenchyma cells are radially elongated, as found here and also by Tuckey and Young *(21)*, only their minimal dimensions would appear in Smith's sections. An additional difficulty with preparation of sections from large-celled tissues arises from mechanics of sectioning. Cell structure is distorted by paraffin compression *(15)* and spatial relationships of large cells and intercellular spaces cannot be accurately revealed in sections prepared from paraffin-embedded tissue. In spite of these problems, Smith's findings *(17, 18, 19)* suggest practical applications for histological background in investigations on growth and storage of apples.

Gas content and its changes in stored apples have received increased attention in studies on quality of apples in storage *(2, 5, 6, 10)*. Changes in gas composition accompany other changes related to structure and texture in stored apples. Bigelow *et al. (1)* found that gas content increased while specific gravity decreased with mealiness and maturity in apples. Hulme *(6)* more recently described special apparatus for gas analyses of apples. Since internal atmosphere, physiological processes, and structure (including total volume of intercellular space) are so intimately related to texture and other fruit qualities, it would seem that more comprehensive investigations are required for further elucidation of problems in both storage and processing of apples.

ADDENDUM

After this paper had been sent to the printer three additional publications on apples were called to the author's attention.[k] These report the results of investigations on the cellular characteristics and physiology of fruit growth. Variation in fruit size, in a variety, was found mainly to be related to number rather than size of cells. Cell enlargement continued throughout fruit life on the tree. More protein nitrogen was characteristic of larger cells. Only low correlations of respiration per cell with cell volume and with protein nitrogen were obtained.

SUMMARY

Measurements were made of flesh parenchyma cells at 2 levels beneath the skin in the mid-regions of 5 varieties of apples. Diameters of outer flesh cells (at ⅛- to ¼-inch depth) and inner flesh cells (about ½-inch depth) averaged, respectively, in microns, as follows: Delicious, 161 and 170; Gravenstein, 221 and 235; and about 190 and 210 for Newtown Pippin, Rome Beauty, and Winesap. The flesh cells tended to be spherical while the core parenchyma cells were elongated and averaged larger in size for all varieties.

[k] Bain, Joan M., and Robertson, R. N. The physiology of growth of apple fruits. I. Cell size, cell number, and fruit development. *Austr. Jour. Sci. Res. Series B.4*, 75-91 (1951).

Robertson, R. N., and Turner, J. F. The physiology of growth of apple fruits. II. Respiratory and other metabolic activities as functions of cell number and cell size in fruit development. *Austr. Jour. Sci. Res. Series B.4*, 92-107 (1951).

Pearson, Judith A., and Robertson, R. N. The physiology of growth of apple fruits. IV. Seasonal variation in cell size, nitrogen metabolism and respiration in developing Granny Smith apple fruits. *Austr. Jour. Sci. Res. Series B.6*, 1-20 (1952).

Percentage of flesh tissue occupied by intercellular spaces averaged between 20 and 22% for Delicious, Newtown Pippin, and Winesap, slightly over 25% for Gravenstein, and 23 to 24% for Rome Beauty. In all varieties the intercellular spaces of the flesh tissue ranged greatly in dimensions, from about 250 to over 600 μ in length, and tended to be elongated radially from the fruit axis.

Amount of cell-wall surface per cubic centimeter was derived from data on cell size and intercellular volume; the lowest amount was found in Gravenstein (190 cm.^{2}) and the highest in Delicious (283 cm.^{2}). The other 3 varieties averaged about 230 cm.^{2} cell-wall surface per cubic centimeter of inner flesh.

Although no consistent correlations between these structural features and saucing rates were shown by the 5 varieties studied, some relationship was apparent. For example, the Gravenstein, a saucing apple that ripens in late summer, has larger cells, more intercellular space, and less cell-wall surface than any of the other varieties. Newtown Pippin and Winesap, both fall varieties relatively resistant to saucing, are similar in these characteristics to Rome Beauty, a late summer or fall apple that sauces nearly as readily as Gravenstein. On the other hand, Delicious, an early fall variety intermediate in saucing quality, has smaller cells and more cell-wall surface than any of the other 4 apples. The lack of consistent relationship between these structural distinctions and saucing supports the view that composition of middle lamella materials between joining cell walls is of primary importance in texture quality.

The open tissue structure of apples has some bearing upon both fresh storage and processing treatments. The large, radially elongated intercellular spaces permit control of internal atmosphere in storage and provide a tissue structure which can be readily impregnated with sirups. More complete information on cellular structure of apples also may aid in elucidation of other problems in the growing and processing of apples.

Acknowledgment

Appreciation is expressed for helpful suggestions and comments by Dr. Benjamin Makower of this laboratory.

LITERATURE CITED

1. Bigelow, W. D., Gore, H. C., and Howard, B. J. Studies on apples. I. Storage, respiration and growth. II. Insoluble carbohydrates of marc. III. Microscopic examinations of apple starch. U. S. Dept. Agr., Bur. of Chem. *Bull. 94* (1905).
2. Denny, F. E. Gas content of plant tissue and respiration measurements. Contrib. Boyce Thompson Inst., **14**, 257 (1946).
3. Foster, A. S., *Practical Plant Anatomy.* 1949, D. Van Nostrand Co., New York.
4. Guadagni, D. G., Sirup treatment of apple slices for freezing preservation. *Food Technol.*, **3**, 404 (1949).
5. Hackney, F. M. V. Studies in the metabolism of apples. VI. Preliminary investigations on the respiration of sliced apple tissue. *Proc. Linn. Soc. N. S. Wales* **70**, 333 (1945).
6. Hulme, A. C. Apparatus for measurement of gaseous conditions inside an apple fruit. *J. Exp. Bot.*, 2, 65 (1951).
7. Kraus, E. J. Variations in internal structure of apple varieties. Oregon Agr. Exp. Sta. *Bull. 135* (1916).

8. LEWIS, F. T. The typical shape of polyhedral cells in vegetable parenchyma and the restoration of that shape following cell division. *Proc. Amer. Acad. Arts & Sci.*, **58**, 537 (1923).

9. LEWIS, F. T. A geometric accounting for diverse shapes of 14-hedral cells; the transition from dodecahedra to tetrakaidecahedra. *Am. J. Bot.*, **30**, 74 (1943).

10. MAGNESS, J. R. Composition of gases in intercellular spaces of apples and potatoes. *Bot. Gaz.*, **70**, 308 (1930).

11. MARVIN, J. W. The shape of compressed lead shot and its relation to cell shape. *Amer. J. Bot.*, **26**, 280 (1939).

12. MATZKE, E. B. The three-dimensional shapes of bubbles in foams. *Proc. Nat. Acad. Science* (U.S.), **31**, 281 (1945).

13. MEYER, B. S., AND ANDERSON, D. B., *Plant Physiology*. 1949, D. Van Nostrand Co., New York.

14. MYLNE, ANN M., AND SEEGMILLER, C. G. Laboratory studies on factors affecting leaching losses during the processing of apples. *Food Technol.*, **4**, 43 (1950).

15. REEVE, R. M. Rapid preparation of thick sections of fleshy plant tissues. *Stain Technol.*, **27**, 29 (1952).

16. REEVE, R. M., AND LEINBACH, R. L. Histological investigations relating to texture in apples. I. Composition and influence of heat on structure. (*Food Research*, this issue.)

17. SMITH, W. H. Anatomy of the apple fruit. Gt. Brit. Dept. Sci. and Ind. Res. Food Inv. Bd. Report *(1935)*, 139 (1936).

18. SMITH, W. H. The histological structure of the flesh of the apple in relation to growth and senescence. *J. Pom. Hort. Sci.*, **18**, 249 (1940).

19. SMITH, W. H. Cell-multiplication and cell-enlargement in the development of the flesh of the apple fruit. *Ann. Bot.*, N. S. **14**, 23 (1950).

20. TETLEY, U. The morphology and cytology of the apple fruit, with special reference to the Bramley's Seedling variety. *J. Pom. Hort. Sci.*, **9**, 278 (1931).

21. TUCKEY, H. B., AND YOUNG, J. ORAN. Gross morphology and histology of developing fruit of the apple. *Bot. Gaz.*, **104**, 3 (1942).

22. WAUGH, A. E. Elements of statistical method. 2nd Ed., 1943, McGraw-Hill Book Company, Ltd., New York.

23. WEIER, T. E., AND STOCKING, C. R. Histological changes induced in fruits and vegetables by processing. *Advances in Food Research. II*, 1949, (edited by E. M. Mrak and G. F. Stewart), Academic Press, Inc., New York.

NUTRITIVE VALUE OF CENTRAL AMERICAN CORNS.

IV. THE CAROTENE CONTENT OF THIRTY-TWO SELECTIONS OF GUATEMALAN CORN[a]

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The pro-vitamin A activity of corn *(Zea mays)* is of considerable importance to Central America. Dietary surveys and clinical and laboratory studies *(7, 8)* indicate that vitamin A deficiency may be widespread. In some groups of people 75% of the diet by weight may consist of corn. The significantly higher serum carotene levels observed in Guatemalan school children compared to those of El Salvador have been attributed to the relatively high carotene content of the yellow corns popular in Guatemala, in contrast to the very low carotene content of the white corns commonly used in El Salvador *(8)*. Because of its practical importance in the local diet, the analysis of 32 varieties of Guatemalan corn for their β-carotene content was undertaken.

Since there is ample evidence that the vitamin A activity of corn is quite different from either the content of total carotenoids or the β-carotene value as measured by routine food analyses techniques, the biological vitamin A activity of one of the varieties was estimated for comparison purposes using chick growth assay.

MATERIALS AND METHODS

Of the 32 corn varieties of different origins 23 varieties including a cross between a Guatemalan and a United States commercial hybrid were planted at an altitude of approximately 5,000 feet in Antigua, Guatemala, during 1947, 1948 or 1949. The characteristics, origin, and sampling of these corns and those of the variety TGY have been described previously *(5)*. An additional 8 collections of corn labeled INCAP were harvested at Santa Maria Cauque, Department of Sactepequez, during the fall of 1950 in fields with an average altitude of 6,500 feet. These corns were all open polinated.

The concentration of β-carotene was measured at 440 mμ in an Evelyn Colorimeter after extraction, purification, adsorption, and elution of the pigment with 5% acetone in petroleum ether on a celite-magnesium oxide column. The method is essentially that of Guilbert *(6)* as modified by Peterson *et al. (11)*. All carotene values cited are the average of two determinations and are expressed on the basis of 10% moisture content.

[a]A cooperative study in which 24 of the corn samples and data relating to their origin were furnished by Dr. Irving E. Melhus, Director, Iowa State College Tropical Research Center, Antigua, Guatemala. The project was aided by a research grant from the Instituto de Fomento de la Producción (INFOP) Guatemala, Central America. Contribution I-25 from the Instituto de Nutrición de Centro América y Panamá.

Quackenbush *(12)*, in a preliminary study by chemical fractionation methods, (Table 1) explored the relationship between total carotenoids as measured at 468 mμ, β-carotene, cryptoxanthol, and other pigments in 4 corn samples reported here.

From the data of Table 1, it was calculated that the 2 samples of TGY contained 6.24 and 4.98 mcg. of vitamin A activity per g. This variety was assayed using 2 chick trials of 5-week duration. New Hampshire straight run baby chicks, 3 days old,

TABLE 1

Carotenoid fraction analysis of three varieties of Guatemala corn [b]

Corn variety	β-carotene	Cryptoxanthol	Luteol	Zeaxanthol	Unidentified
	mcg./g.	*mcg./g.*	*mcg./g.*	*mcg./g.*	*mcg./g.*
TGY[c]	2.94	6.61	2.45	5.28	0.74
TGY	2.04	5.88	1.03	3.92	4.68
12A–46	0.20	0.96	2.43	1.50	0.95
15A–46	0.25	0.27	.....	0.14	.....

[b] Data furnished by F. W. Quackenbush from analysis carried out at Purdue University.
[c] Tiquisate Golden Yellow.

were banded and distributed by weight among the various experimental groups. The chicks were housed in special, electrically heated, all-wire cages with raised screen bottoms. Feed and water were provided *ad libitum* and weekly individual weights were obtained.

In experiment 1, the baby chicks were fed an all-vegetable protein basal ration. In experiment 2, a vitamin test casein was added to increase the growth rate of the chicks. These rations were as follows:

Basal Ration, Experiment 1		Basal Ration, Experiment 2	
	g.		*g.*
Corozo oil meal	40	Corozo oil meal	15
Sesame oil meal	12	Sesame oil meal	30
Cottonseed oil meal	12	Cottonseed oil meal	5
Sorghum	32.3	Casein VT	4
		White corn	42.3

Both rations also contained the following: bone meal, 1 g.; $CaCO_3$, 1 g.; NaCl with 4% of $MnSO_4$, 1 g.; Delsterol, 200 mg.; choline, 125 mg.; thiamine, 0.2 mg.; riboflavin, 0.35 mg.; pantothenic acid, 1.2 mg.; nicotinic acid, 1.5 mg.; and pyridoxine, 0.35 mg.

The sorghum in experiment 1 and the white corn in experiment 2 were replaced with corresponding amounts of the corn variety TGY as indicated in Table 4.

RESULTS

The β-carotene concentrations found in the 24 varieties of Guatemala corn are given in Table 2. Since some samples contained seeds that varied in color, the color listed is the prevailing color of the seed lot. The β-carotene in the yellow varieties ranged from 0.020 to 0.210 mg. per 100 g. with an average of 0.115 (s 0.062). The white corn selections grown at Antigua varied in β-carotene content from 0.010 to 0.096 mg. per 100 g. with an average of 0.033 (s 0.024). In the collections of INCAP the range for yellow and deep red hull and yellow endosperm was 0.131 to 0.177 mg. per 100 g., with an average of 0.157, and for the white and black hulled corns was 0 to 0.024 mg. per 100 g., with a mean value of 0.014 mg. per 100 g. The β-carotene content of the deep yellow variety TGY grown in 5 different localities, Table 3, ranged from 0.180 to 0.380 mg. per 100 g. with an average of 0.300 mg. per 100 g.

For the biological assays, the number of chicks, their mortality, weight gain, and feed efficiency are presented in Table 4. Comparison of the growth rates of the chicks fed various levels of corn and standard vitamin A indicates that the samples of TGY contained approximately 800 I. U. of vitamin A per 100 g. in the first trial and 1,250

TABLE 2

The β-carotene content of 32 varieties of corn

Grown at Antigua, Guatemala									Grown at Sta. Maria Cauque, Guatemala		
Variety No.		Carotene	Variety No.		Carotene	Variety No.		Carotene	Variety No.		Carotene
		mg./100 g.			*mg./100 g.*			*mg./100 g.*			*mg./100 g.*
206–44 ⊗	W	0.038	166–44 O.P.	W	0.010	92–44 O.P.	W	0.027	INCAP 18	Y	0.151
159–44 (2s)	Y	0.159	31–44 #	Y	0.058	129A–46 #	Y	0.085	INCAP 19	W	0.023
47A–46 O.P.	W	0.019	20–47 ⊗	Y	0.092	12A–46 # ⊗	Y	0.172	INCAP 22	B	0.007
1470–45 #	W	0.047	1483–45 ⊗	W	0.018	TGY	Y	0.162	INCAP 409	B	0.024
25A–46 ⊗	Y	0.027	21A–46B #	W	0.096	1626–45 O.P.	W	0.018	INCAP 416	W	0.000
92A–46 # ⊗	Y	0.020	10A–46 O.P.	Y	0.210	7A (WF9×38–11)	Y	0.194	INCAP 417	r	0.169
192–44 # ⊗	Y	0.109	142–48 O.P.	Y	0.135	26A–46⊗	W	0.016	INCAP 418	R	0.177
15A–46 O.P.	Y	0.070	118A–46 #	W	0.032	200–47 O.P.	W	0.046	INCAP SMC	Y	0.131

Legend:
W = white kernel
Y = yellow kernel
B = black hulled kernel
r = light red hulled kernel
R = dark red hulled kernel

I. U. in the second. This agrees satisfactorily with the chemical estimation of Quackenbush of 1,040 I. U. of vitamin A activity per 100 g. (6.24 mcg./g.) and 830 I. U. (4.98 mcg./g.) for 2 other samples of this variety. The mortality rates and clinical evidence of avitaminosis A in the chicks fed at the higher levels of both corn and vitamin A standard give added support to the growth data.

TABLE 3

β-Carotene content of the variety TGY grown in five different localities

Sample No.	Location	Altitude	Content
		feet	*mg./100 g.*
INCAP–271	Coatepeque	100	0.380
INCAP–275	Cuyuta	100	0.350
INCAP–266	Tiquisate	150	0.300
INCAP–298	Retalhuleu	200	0.290
INCAP–276	Antigua	4,953	0.180

DISCUSSION

The values reported for the β-carotene content of the corns are not the full measure of their pro-vitamin A activity. This is due to the fact that the method for β-carotene does not measure the cryptoxanthol present although it is commonly considered to be approximately half as active biologically as β-carotene. On the other hand when total carotenoids are measured other pigments absorbed in the same region of the spectrum are credited with a vitamin A activity they do not possess.

The value of estimating the vitamin A activity of corn by the chemical fractionation of the total carotenoid present is demonstrated by the chick assay data. They confirm the total pro-vitamin activity of the variety TGY as estimated by the chemical method of Quackenbush when cryptoxanthol is calculated to have one-half the pro-vitamin A activity of β-carotene.

The influence of environmental factors, including soils, on the β-carotene content of corns is suggested by the data presented for one lowland variety, Tiquisate Golden Yellow (TGY), which was grown in 4 different lowland areas and one highland area. The data indicate that the β-carotene content of this lowland corn was reduced approximately 48 to 53% when it was grown at 5,000 feet. The higher value for this corn when grown in the lowland as compared to the highland location may be related to its better adaptation to the former locality.

Although environmental factors are important, the pro-vitamin A activity of corn is also greatly influenced by some of the factors that control the development of yellow endosperm. Mangelsdorf and Fraps *(10)* showed that there is a direct quantitative relationship between the amount of pro-vitamin A and the number of genes for yellow pigment. These findings were confirmed by Kemmerer *et al. (9)* who stated that the number of genes present for yellow color in corn directly affects the amount of vitamin A-active carotenoid pigment, but does not affect appreciably the relative proportions of alpha- and beta-carotene and cryptoxanthol. Randolph and Hand *(13)* showed that doubling the number of chromosomes in pure yellow corn caused a 40% increase in the carotenoid content of corn grain.

Until relatively recently corn breeders were concerned mainly with obtaining high yields and resistance to disease and drought. Aurand *et al. (3, 4)* have shown that when attention is given to both yield and carotene, certain high yielding corn lines show relatively high carotene content. Since possibilities exist for the selection of corns of higher pro-vitamin A content *(14, 15, 16)*, as well as yield, the determination of β-carotene and cryptoxanthol is also of potential value to the Agricultural Experiment Stations in Central America that wish to develop superior corn varieties.

TABLE 4

Growth and mortality of baby chicks supplemented with a vitamin A standard and different percentages of yellow corn (TGY)

Addition to ration	Number of chicks		Average weight		Feed efficiency[f]	Number chicks with avitaminosis A
	Start	End	Start	End		
Basal Ration 1, Experiment 1						
			grams			
Check	12	0	39	....	....	....
Corn 1.25%[d]	12	2	39	164	....	2
Vitamin A, 20 I. U.	12	3	39	213	....	1
Corn, 2.5%[d]	12	8	39	222	2.87	8
Vitamin A,40 I. U.	12	8	39	238	2.78	1
Corn, 5.0%	12	8	39	253	2.72	1
Vitamin A, 80 I. U.	12	10	39	230	2.51	0
Corn, 10.0%	12	10	39	238	2.69	0
Basal Ration 2, Experiment 2						
Check	12	2	39	153	....	2
Corn, 1.0%	12	7	39	285	....	7
Vitamin A, 25 I. U.	12	8	39	320	....	6
Corn, 2.0%	12	7	39	305	....	3
Vitamin, A, 50 I. U.	12	12	39	382	2.21	2
Corn, 4%	12	12	39	343	2.47	3
Vitamin A, 100 I. U.	12	12	39	394	2.14	0
Corn, 8%	12	12	39	375	2.51	0

[d] Tiquisate Golden Yellow (TGY), substituted for the ground sorghum or white corn of the basal diets.
[e] U. S. P. vitamin A standard, added to each 100 g. of the basal diet.
[f] Grams fed to produce one gram of gain.

In Mexico, Central America, Panama, and many areas of South America, human diets are such that it is of the utmost importance that corns with good nutritional value as well as superior agronomic qualities be produced. In Guatemala the rural population depends upon corn for their principal food and consumes quantities as large as 500 g. of corn per person per day *(8)*. Where yellow corn is used, its vitamin A activity is of concern since the most common human vitamin deficiency appears to be that of vitamin A *(8)*.

It seems apparent from this preliminary work that those yellow corns reported to be superior in other qualities *(1, 2, 5)* should be screened by

either chemical or biological techniques for their vitamin A activity. Such analyses are also necessary for the correct evaluation of dietary surveys in areas where yellow corn is an important part of the diet.

SUMMARY

The β-carotene content of 32 different Guatemalan corns *(Zea mays)* from 2 general areas was determined. In the Antigua area, 13 yellow varieties averaged 0.115 (s 0.062) and 11 white varieties averaged 0.033 (s 0.024) mg. β-carotene per 100 g. In the Santa Maria Cauque area 4 yellow to red varieties averaged 0.157 and 4 white to black varieties averaged 0.014 mg. β-carotene per 100 g.

One lowland variety, Tiquisate Golden Yellow (TGY), grown in 4 lowland locations (100-200 feet) averaged 0.330 mg. β-carotene per 100 g. and when grown in one highland area (approximately 5,000 feet) averaged 0.180 mg. β-carotene per 100 g.

The vitamin A activity of 2 samples of TGY grown in the lowlands was estimated by chick growth assay to be 0.50 and 0.62 mg. per 100 g. Since the β-carotene content accounts for less than half of this activity, it is apparent that the analysis of corn for β-carotene alone is not a satisfactory means of appraising its pro-vitamin A content. Evidence is presented that most of the activity not accounted for by β-carotene is due to cryptoxanthol.

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LITERATURE CITED

1. Aguirre, F., Robles, C. E., and Scrimshaw, N. S. The nutritive value of Central American Corns. II. Lysine and methionine content of twenty-three varieties in Guatemala. *Food Research*, **18**, 268 (1953).
2. Aguirre, F., Bressani, R., and Scrimshaw, N. S. The nutritive value of Central American Corns. III. Tryptophane, niacin, thiamine, and riboflavin content of twenty-three varieties in Guatemala. *Food Research*, **18**, 273 (1953).
3. Aurand, L. W., Miller, R. C., and Huber, L. L. The influence of heredity on the carotene content of corn. *Science*, **106**, 493 (1947).
4. Aurand, L. W., Miller, R. C., and Huber, L. L. Influence of heredity on carotene and protein contents of corn. The Pennsylvania State College, Agr. Expt. Sta. State Coll. *Bull. 526* (1950).
5. Bressani, R., Arroyave, G., and Scrimshaw, N. S. The nutritive value of Central American corns. I. Nitrogen, ether extract, crude fiber, and minerals of twenty-four varieties in Guatemala. *Food Research*, **18**, 261 (1953).
6. Guilbert, H. R. Determination of carotene as a means of estimating the vitamin A value of forage. *Ind. Eng. Chem., Anal. Ed.*, **6**, 452 (1934).
7. Guzmán, M., and Scrimshaw, N. S. Serum ascorbic acid, vitamin A, carotene, vitamin E, riboflavin, and alkaline phosphatase values in Central American school children. *Fed. Proc.*, **11**, 445 (1952).
8. Instituto de Nutrición de Centro América y Panamá. (Unpublished data.)
9. Kemmerer, A. R., Fraps, G. S., and Mangelsdorf, P. C. The relation between the vitamin A active carotenoids in corn and the number of genes for yellow color. *Cereal Chem.*, **19**, 525 (1942).
10. Mangelsdorf, P. C., and Fraps, G. S. A direct quantitative relationship between vitamin A in corn and the number of genes for yellow pigmentation. *Science*, **13**, 241 (1931).

11. PETERSON, W. J., HUGHES, J. S., AND FREEMAN, H. F. Determination of carotene in forage. A modification of the Guilbert method. *Ind. Eng. Chem., Anal. Ed.*, **9**, 71 (1937).

12. QUACKENBUSH, F. W. (Personal communication, 1949).

13. RANDOLPH, L. F., AND HAND, D. B. Relation between carotenoid content and number of genes per cell in diploid and tetraploid corn. *J. Agr. Research*, **60**, 51 (1940).

14. SADANA, J. C., AND AHMAD, B. The carotenoid pigments of local varieties of yellow maize. *Indian J. Med. Research*, **34**, 59 (1946).

15. SCOTT, G. C., AND BELKENGREN, R. O. Importance of breeding peas and corn for nutritional quality. *Food Research*, **9**, 371 (1944).

16. WHITE, J. W., BRUNSON, A. M., AND ZSCHEILE, F. P. Studies on the carotenoids. V. Spectrophotometric determination of the carotenoids of yellow corn grain. *Ind. Eng. Chem., Anal. Ed.*, **14**, 778 (1942).

CORRECTED NORMAL AND CHI-SQUARE APPROXIMATIONS TO THE BINOMIAL DISTRIBUTION IN ORGANOLEPTIC TESTS

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In papers on organoleptic testing the normal distribution and the chi-square distribution, with one degree of freedom, are frequently used to approximate probabilities of the binomial distribution. In these papers no consideration has been given to the fact that the binomial distribution is discrete while the normal and chi-square distributions are continuous. Suitable corrections for continuity are discussed in many places *(1, 2, 5, 6)*.

In order to correct for discreteness in using the normal curve approximation, 5 correct selections should be considered as occupying an interval ranging from 4.5 up to 5.5; 8 correct choices, an interval ranging from 7.5 to 8.5; and so on. The probability of 8 or more correct choices would be represented by the area under the normal curve from 7.5 to infinity. The approximate normal deviate expressed in terms of the standard deviation is then

$$z = \frac{(X\text{-}m) - 0.5}{\sigma} = \frac{(X\text{-}np) - 0.5}{\sqrt{npq}}$$

where X is the number of correct selections in n trials, p is the probability of a correct selection in any one trial, and $q = 1 - p$.

A similar correction for the chi-square distribution consists in subtracting 0.5 from the value of *(o-e)* so that the corrected value is

$$\chi^2 = \Sigma \frac{[(o\text{-}e) - 0.5]^2}{e} = \frac{[(X\text{-}np) - 0.5]^2}{npq}$$

where o and e represent the observed and expected frequencies. The expected chance probability of any number (or more) of correct selections is determined on the basis of one tail or one side of the distribution. Since the usual chi-square test involves both tails of the distribution, the tabular probabilities are for a 2-tailed test, and care must be exercised when using the tables to determine probabilities for a one-tailed test. The appropriate probability for the one-tailed case can be determined from the following rules:

(a) If (X-np) — 0.5 is positive, the required probability is ½ that shown in the usual χ^2 table.

(b) If (X-np) — 0.5 is negative, the required probability is 1 minus ½ that shown in the usual χ^2 table.

The appropriateness of one- and 2-tailed tests in organoleptic testing will be discussed in another paper.

For one degree of freedom χ^2 is equal to z^2. The corrections have the effect of reducing the values of both in a similar way, so that the corrected χ^2 is still equal to the corrected z^2. Unless a correction to adjust for the

TABLE 1

True probabilities from the binomial distribution and corrected and uncorrected approximations from the normal or chi-square distributions

n	p	Number of successes	16	15 or more	14 or more	13 or more	12 or more	11 or more	10 or more	9 or more	8 or more	7 or more
16	½	True probability	.000	.000	.002	.011	.038	.105	.227	.402	.598	.773
		Corrected approximations	.000	.001	.003	.012	.040	.106	.227	.401	.599	.773
		Uncorrected approximations	.000	.000	.001	.006	.023	.067	.159	.308	.500	.692
16	⅓	True probability	.000	.000	.000	.000	.001	.004	.016	.050	.126	.292
		Corrected approximations	.000	.000	.000	.000	.001	.003	.014	.048	.125	.268
		Uncorrected approximations	.000	.000	.000	.000	.000	.001	.007	.026	.079	.188

n	p	Number of successes	10	9 or more	8 or more	7 or more	6 or more	5 or more	4 or more	3 or more	2 or more	1 or more
10	½	True probability	.001	.011	.055	.172	.377	.623	.828	.945	.989	.999
		Corrected approximations	.002	.013	.057	.171	.376	.624	.829	.943	.987	.998
		Uncorrected approximations	.001	.006	.029	.103	.265	.500	.736	.897	.971	.994
10	⅓	True probability	.000	.000	.003	.020	.076	.210	.441	.701	.896	.983
		Corrected approximations	.000	.000	.003	.017	.073	.217	.455	.712	.891	.971
		Uncorrected approximations	.000	.000	.001	.007	.037	.132	.328	.589	.814	.941

n	p	Number of successes						5	4 or more	3 or more	2 or more	1 or more
5	½	True probability						.031	.188	.500	.812	.969
		Corrected approximations						.037	.187	.500	.813	.963
		Uncorrected approximations						.012	.090	.326	.674	.910
5	⅓	True probability						.004	.045	.210	.539	.868
		Corrected approximations						.004	.041	.215	.563	.866
		Uncorrected approximations						.001	.014	.103	.375	.736

lack of continuity is made, the resulting probabilities are in error and the error increases as the number of tests is reduced.

Peryam and Swartz state *(4)* that as n, the total number of judgments, "is reduced, the error arising in both the chi-square and in the Critical Ratio (normal curve) method becomes progressively larger and neither should be used when n is less than 16. Below this point the exact probabilities should be calculated by the binomial theorem."

By correcting for continuity the number of tests can safely be reduced. In Table 1 are shown for $n = 5$, $n = 10$, and $n = 16$ and $p = \frac{1}{2}$ (paired tests) and $p = \frac{1}{3}$ (triangle tests), the true *(3)* and expected probabilities calculated from the normal or chi-square distributions.

From the table it is apparent that the probabilities as determined from the corrected normal or chi-square distributions are very close to the true values obtained from the binomial distribution and give good approximations for as low as 5 trials. The uncorrected values do not approximate the true values nearly as closely. The corrected values should be used in all tests involving one degree of freedom.

SUMMARY

In using the normal curve and chi-square (with one degree of freedom) distributions to approximate probabilities of the binomial distribution, the correction for continuity should always be made. In using chi-square, the appropriate value is

$$\text{Corrected } \chi^2 = \frac{[(X\text{-}np) - 0.5]^2}{npq}$$

and in using the normal curve approximation, the proper normal deviate is

$$z = \frac{(X\text{-}np) - 0.5}{\sqrt{npq}}$$

where X represents the number of successes in n trials, p the probability of success in a single trial, and $q = 1 - p$.

Failure to use these corrected values in determining the probability of success results in probabilities that underestimate those of the binomial through practically the whole range of frequencies.

LITERATURE CITED

1. Fisher, R. A. *Statistical Methods for Research Workers.* 10th ed., 1946, Oliver and Boyd, Edinburgh.
2. Goulden, C. H. Methods of Statistical Analysis. 2nd ed., 1952, John Wiley and Sons, New York.
3. *Nat. Bureau of Standards,* Applied Mathematics Series 6. Tables of the binomial probability distribution. 1950, U. S. Gov't Printing Office, Washington, D. C.
4. Peryam, David R., and Swartz, VeNona, W. Measurement of sensory differences. *Food Technol.,* **4**, 390 (1950).
5. Snedecor, G. W. *Statistical Methods.* 4th ed., 1946, The Iowa State College Press, Ames, Iowa.
6. Yates, F. Contingency tables involving small numbers and the χ^2 test. *J. Roy. Stat. Soc. Suppl.,* **1**, 217 (1934).

PREVENTION OF MICROBIAL GROWTH AT INTERFACE SURFACES BETWEEN LIQUID AND SOLID SUBSTRATES

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Wherever liquid substrates that are susceptible to development of microorganisms come in contact with solid surfaces, especially at those peripheral areas or zones that are also exposed to the atmosphere, the rate of growth of microorganisms is greatly accelerated in those specific areas. On account of this accelerated growth sanitary measures at these specific areas become vitally important; at the same time they are difficult to apply with complete effectiveness in food plants and particularly those plants devoted to the fermentation industries. Before the introduction of stainless steel equipment in food and beverage plants, and before the use of high grade steel in place of the old wooden fermenters with which the early distilleries were equipped, the problem of sterilizing these units, was an insuperable one. In consequence, the control of fermentation was very imperfect.

Even where monel metal or polished surfaces are provided in storage tanks or fermenters in food or beverage industries, the surfaces of the metal in contact both with the atmosphere and the liquid substrate are highly vulnerable to microbial development. Hence, the use of detergents and surface-cleaning agents to eliminate contamination from beneath shallow layers of scale or incrustation is a very essential technique.

There are, of course, potential zones of accelerated growth in addition to the surface interfaces between liquid and solids. The same vulnerability exists in the submerged surfaces of the liquid substrate in contact with the sides or the bottom of tanks or vats or fermentation vessels. The difference is that in the latter category the conditions promote the growth of anaerobic bacteria, whereas the upper marginal exposures tend to support aerobic bacteria and mould fungi.

The tendency of microorganisms to colonize at the liquid-solid interfaces of tanks and storage vessels is actually a minor problem in contrast with the problem of preventing such colonization in water during filtration over activated carbon. The surface of carbon offers such a tremendous area for colonization that the zones of growth become magnified immeasurably. Braidich has pointed out that one cubic inch of the type of carbon used for water purification represents a composite surface of more than 20,000 square yards or approximately 5 acres in area. It is on these surfaces that bacteria find conditions admirably suited for colonization; not only are they protected from the accumulation of the toxic products of their own metabolism, but also food and nutrients are adsorbed on these surfaces, constituting a convenient source of supply to the colonies of bacteria developing at those areas.

This stimulating effect of finely divided colloidal material upon microbial development has long been known. Sohngen *(6)* over three decades

ago, described the accelerative effects of bone char, finely divided clay, and paper upon the fermentative activities of yeast. Abderhalden and Fodor *(1)* made similar studies, their results confirming the published findings of the previous author. More recently Owen and Denson *(3)* studied the accelerative action of activated carbon and cellulose fiber upon the rate of fermentation and the cell development of *Saccharomyces cerevisiae;* they found that all of these substances acted as catalysts in speeding the rate of fermentation and cell development of the microorganims studied.

Moreover, the carbon surfaces are particularly favorable for the growth and development of those species of bacteria that are notorious for their nuisance value in producing unpleasant odors in water. Many of these species of *Streptomyces (actinomycetes)* whose natural habitat is in water supplies tend to form colonies in water, producing musty odors and tastes that greatly impair the quality of a water supply. In beverage plants where the filtration of plant water is a necessity for the purpose of removing any excess of chlorine, it is the common experience that the water coming from these purifiers contains more bacteria per cc. than did the original water prior to filtration and the dechlorination incident thereto. This phenomenon is the result of the combined effects of chlorine removal and acceleration by surface contact with the carbon particles.

MODE OF PROCEDURE FOR PREVENTION

There has been the greatest need for an effective means of rendering activated carbon used for the dechlorination of water supplies bacteriostatic. If this could be accomplished the build-up of bacteria in the water during its filtration might be avoided. Moreover the dechlorination potential of the carbon would be unaffected. The writer *(4)* has been engaged in such an investigation for the past few years, and much progress has been made towards the attainment of this very desirable end.

The difficulty has been that in the choice of an impregnating agent to induce bacteriostasis in a carbon the product chosen must comply with 3 essential requirements, viz., (a) it must be non-toxic to the human system when used in the maximum amounts that would possibly be discharged into the water supply; (b) it must be relatively low in price; (c) it must be effective over fairly long periods of time. For our purposes, a relatively non-toxic inorganic material was chosen. Its effectiveness may be seen in Figures 1 and 2 showing bacterial counts in the effluent of a plant filter. It will be noted that the count in the untreated plant purifier is in every instance higher than in the influent to the plant filter.

The mode of procedure for impregnating the activated carbon with an effective bacteriostatic agent is preferably to incorporate the dry salt in the combustion chamber when the carbon is being activated or when the organic material is being carbonized. However, our tests have been principally confined to the use of activated carbons which have been impregnated in our laboratory subsequent to their manufacture. This impregnation has been very easily achieved, merely by percolating the solution of the bacteriostatic agent through a column of the activated carbon, and then testing the carbon to determine how much of the agent has been adsorbed from the solution. It is sometimes necessary to recirculate the solution of the impregnating agent over the carbon to be treated in order to attain an effective concentration of the agent on the surfaces of the carbon. The treated carbon is then dried, sterilized in the autoclave at 15 pounds' pressure for 45 minutes, cooled, and then transferred to pyrex glass cylinders, used as filters. These filtering units were set up as a battery for testing the efficacy of the bacteriostatic carbons. Each cylinder was 20 inches in length and 3 inches in inside diameter.

The cylinders, it should be explained, were fitted with brass flanged tops and bottoms and connected at the top with a one-half inch water line from the city water

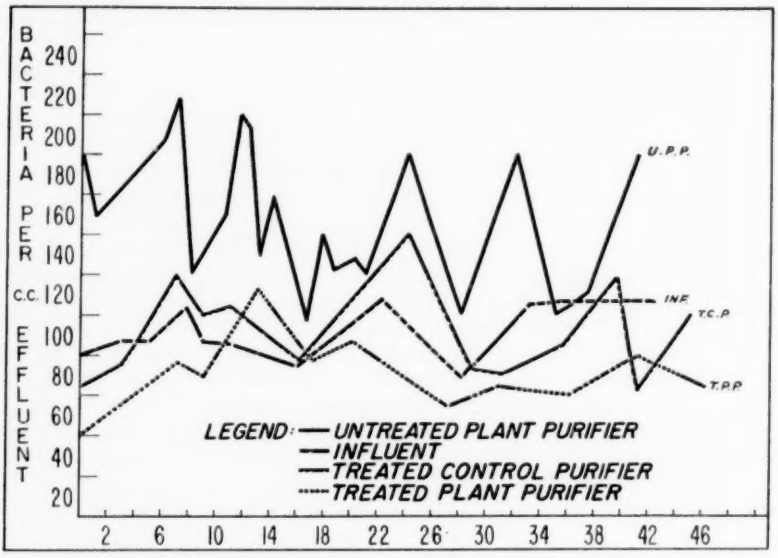

Figure 1. Effectiveness (measured over 48 hours) of bacteriostatic carbon on the control of bacteria in water filtration.

supply. The bed of carbon in each cylinder was supported by a bed of sand and gravel to prevent the leakage of fine particles of carbon into the effluent.

We have impregnated during manufacture an activated filtering material that has been developed for the sugar refining industry *(5)*; its effectiveness in preventing microbial development in the sugar liquors during filtration has been even more conclusive than the results obtained on water filtration from carbons impregnated after manufacture.

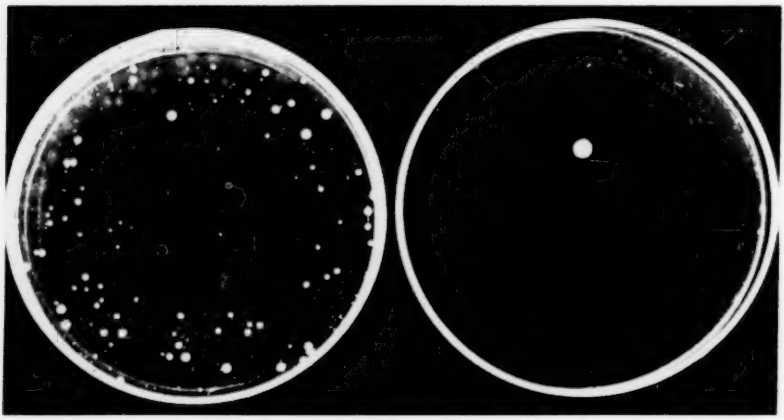

Figure 2. Bacterial colonies developed from water exposed to untreated carbon for 48 hours (left) and from water exposed to treated carbon for 48 hours (right).

The significance of this development of a bacteriostatic carbon for use in the filtration of water or beverages or even pharmaceuticals can hardly be over-emphasized. These products and almost every water supply used in the manufacture of food or beverages have to be purified by the use of carbon filtration. It is most desirable that this end should be achieved without the acquisition of large bacterial populations resulting from the removal of the chlorine by the carbon in the case of chlorinated water supplies, or by the surface acceleration of growth or colonization by bacteria in the case of unchlorinated therapeutical agents.

Additional uses. There is still another example of bacterial colonization in food and beverage plants on exposed surfaces at interfaces of liquid and solid substances. Reference is made to the use of paper and fiber filters for filtration of water through paper disc filters to give the final polish to the water and to remove any traces of residual carbon particles that might be left in the water. Here again the bacterial counts are found to increase as a result of the filtration, and the density of population is greater in the effluent than in the influent of such filters. Although it is more difficult to render these paper fibers bacteriostatic, much evidence has been obtained that suggests that it is not impossible.

As an example of the effectiveness of this treatment on cellulose and asbestos fibers used in industrial fiber filter pads, Table 1 shows the rate of development of bacteria on the surfaces of untreated and treated fibers. The fibers were sterilized in tubes by autoclaving them for 30 minutes at 15 pounds' pressure. Tap water was then added to each tube and the rate of growth in contact with the fibers was determined by plate counts, with results as shown in Table 1.

TABLE 1

Bacterial development on fibers

	24 hours	48 hours	72 hours	96 hours	120 hours
Untreated fibers	3	87	TTC[a]	TTC[a]	TTC[a]
Treated fibers	1	1	4	2	8

[a] TTC = Colonies too numerous to count.

From Table 1 it will be seen that the bacteriostatic treatment of the fibers was effective over a period of five days; the control fibers sustained a development of more than 100 bacteria per cc. by the third day the water was in contact with them.

In another test using wood pulp fibers alone, the count on the water in contact with the untreated fibers reached a level of over 200 per cc. in 48 hours; the treated fibers had only 77 per cc. at the end of that period.

The value of an effective bacteriostatic fiber is clear. Its use in filter pads for water or beverages or for certain therapeutical liquids that are susceptible to microbial growth and contamination would be most advantageous. In the filtration of wines, for example, it is often found that the development of bacteria or yeast and especially acetic acid bacteria on the filters tend to intermittently recontaminate the finished product.

Still other examples of sources of surface acceleration of microbial growth at the interface of solids and liquids are to be found in concrete or cement storage vats, the vessels used for liquids, or fermenters in industrial fermentations, or even the cement walls of cold storage rooms where there is usually a high relative humidity and where moisture frequently gathers and condenses on these surface areas. Not only do concrete surfaces exposed to moisture tend to accelerate the growth of microorganisms, but the growth tends to break down the structure and to shorten the life of the cement. In this connection it is interesting to note that there has been developed in recent years a cement with so-called anti-bacterial properties which is claimed to maintain bacteriostasis at the interface between the cement surface and moisture films attached thereto *(2)*. In dairies and creameries and in food plants in general the use of an anti-bacterial cement for walls and floors should greatly facilitate the maintenance of sanitary conditions throughout the interior and around the premises of factories of this kind.

It would also fill a great need as a protective flooring for washer and locker rooms, around swimming pools, and wherever protection is needed against the development of pathogenic bacteria and fungi.

CONCLUSION

In conclusion it can safely be predicted that one of the important developments of sanitary engineering in the future is likely to be that of impregnating surfaces of walls and floors to render them immune to microbial growth and survival. Considering the accelerative growth of microorganisms at these interfaces of solids and liquids there would appear to be no greater need than this for improved sanitary maintenance of food plants.

LITERATURE CITED

1. Abderhalden, E., and Fodor, F. *Ferment. Forsch. Bd.* **5**, 90-110 (1921).
2. Levowitiz, B. *Am. Milk Review*, Nov. 1952.
3. Owen, W. L., and Denson, W. P. *Cent. Für Bakt. Bd.* **77** (1929).
4. Owen, W. L. *Sugar Journal*, 1952.
5. Owen, W. L. *J. Ind. Eng. Chem.*, **43**, 606 (1951).
6. Sohngen, N. L. *Cent. für Bakt. Abt.* **11**, 38 (1913).

STUDIES ON THE ANAEROBIC DECOMPOSITION OF ASCORBIC ACID

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Oxidation of ascorbic acid by atmospheric oxygen has been studied by many workers, as it is the reaction mainly concerned in the loss of this vitamin during the preparation of processed foods. In canned foods, however, free oxygen is present in restricted amounts at the time of sealing and disappears entirely within one month of canning (Horner, *7*; Feaster, Tompkins, and Pearce, *4*). Nevertheless, ascorbic acid loss in canned foods continues at a steady rate throughout the storage life. This continued loss must be due to an anaerobic destruction of ascorbic acid.

In strongly acid solution ascorbic acid decomposes to furfural and carbon dioxide (Herbert *et al.*, *6*; Lamden and Harris, *11*), and the same reaction may occur more slowly in less acid or neutral products. In the author's investigations, the decomposition was studied in buffer solutions of pH 2.2-6.0. The effect of other substances, known to be present in foods, was also investigated. The preliminary measurements were made at 100°C. to obtain results more rapidly, and subsequently a further series of studies was made at 30°C.

METHODS

One-ml. aliquots of citrate-phosphate[a] buffer with an ascorbic acid concentration of 0.01M (176 mg./100 ml.) were sealed in small glass tubes in the presence of nitrogen or carbon dioxide. The latter was used only for studies at 100°C. where the carbon dioxide, being insoluble, did not affect the pH. Aliquots were analyzed immediately and also after varying periods at 30° and 100°C. Before storage at 30°C. all tubes were pasteurized at 100°C. for 10 minutes.

Each tube was cracked open and the contents washed into a 25-ml. volumetric flask and made to volume with 2% oxalic or 3% metaphosphoric acid. Oxalic acid was used when furfural was determined. Ascorbic acid (uncorrected) was determined by titrating a 5-ml. aliquot with 2,6 dichlorophenol-indophenol. "Apparent ascorbic acid" was determined by titrating another aliquot after condensing the ascorbic acid with formaldehyde (Wokes, Organ, and Jacoby, *12*) and subtracted from the total titre to give true ascorbic acid. All results refer to true ascorbic acid.

Determination of furfural was based on the method of Duncan *(3)*. Another 5-ml. aliquot was diluted to 50 ml. after adding 5 ml. of 2% oxalic acid and 10 ml. of 0.2M Na_2HPO_4. In an Evelyn colorimeter tube were mixed 5 ml. of the diluted solution and 5 ml. of a mixture of one volume of freshly distilled aniline and nine volumes of glacial acetic acid. After standing for an hour in the dark at 20°C. the absorption was measured in the colorimeter, using a 520-filter. The concentration of furfural was obtained from a standard curve.

Determinations were also made of carbon dioxide produced from ascorbic acid after 104 weeks at 30°C. After introducing 25 ml. of buffer containing 0.01M ascorbic acid into a 50-ml. Florence flask, the neck was drawn out and sealed *in vacuo*. After incubation a file mark was made near the tip and the flask placed in a water bath. A

[a] Preliminary experiments gave similar results in phosphate, acetate, and phthalate buffers.

second flask containing 20 ml. of 0.05N $Ba(OH)_2$ was fitted with a rubber stopper and 2 inlet tubes, giving rubber connections to the neck of the other flask and to a vacuum pump. After evacuating, both rubber connections were closed with screw clips and the tip of the first flask broken off through the rubber tubing. On cautiously opening the connection between the 2 flasks carbon dioxide was readily evolved and absorbed by the barium hydroxide. The temperature of the bath was finally raised to 60°C. to ensure complete removal. The carbon dioxide was determined by titration with standard acid.

In studies on any solution containing ascorbic acid a corresponding solution without ascorbic acid was incubated and analyzed at the same time. The results were corrected by subtracting the blank analyses but the correction was usually negligible.

EFFECT OF pH IN PURE BUFFER SOLUTIONS

Since the relation between log (per cent retention) and time was approximately linear down to 25% retention of the original ascorbic acid, the destruction is evidently a first order reaction. The velocity constant k was calculated as min^{-1}. The velocity constants at 30°, 40° (by interpolation), and 100°C. are given in Table 1. For comparison the constants for oxidation of ascorbic acid at 40°C. in phthalate buffers saturated with air and containing 1 p.p.m. of added copper are given in the same table. The data for oxidation were obtained by Huelin and Stephens *(8)*.

TABLE 1

Effect of pH on velocity constant

pH	Velocity constants for anaerobic decomposition at			Velocity constant for oxidation at 40°
	30°	40°	100°	
2.2	8.3×10^{-7}	21.6×10^{-7}	2.3×10^{-4}	0.2×10^{-2}
3.0	9.8	27.1	3.9	1.2
4.0	8.9	26.8	5.8	5.3
5.0	3.6	11.2	2.9	14.4
6.0	1.8	5.9	1.9	25.1

The velocity constants for anaerobic decomposition at 40°C. were calculated by assuming the Arrhenius equation: $d\ \ln k/dT = A/RT^2$, which gives a linear relation between log k and 1/T. At pH 2.2-6.0 the anaerobic decomposition of ascorbic acid is an extremely slow reaction with a velocity constant not more than one thousandth that of the oxidation under optimum conditions. While the copper-catalyzed oxidation increases with increasing pH, the anaerobic decomposition passes through a maximum at pH 3-4. The optimum pH is near the pK_1 of ascorbic acid (4.17), indicating that both the undissociated ascorbic acid and the monovalent ascorbate ion participate in the reaction.

These results can also be expressed in terms of times of half destruction (Table 2), which can be more readily compared with data on the retention of ascorbic acid in canned foods. If $t_{0.5}$ is the time of half destruction, then the fraction remaining after a time $nt_{0.5}$ is given by $(0.5)^n$.

TABLE 2

Effect of pH on time of half destruction

pH	Time of half destruction at		
	30°	32°	100°
	weeks	*weeks*	*hours*
2.2	83	68	50
3.0	70	57	30
4.0	77	61	20
5.0	190	150	40
6.0	390	305	60

The times of half destruction at 32°C.(90°F.), which were obtained by interpolation, are considered in a subsequent section in relation to data on canned foods.

The work of Lamden and Harris *(11)* on the destruction of ascorbic acid in 1-50% citric acid solutions at 100°C. indicates that at this temperature the decomposition increases again as the pH falls from 2 to 1. The non-oxidative nature of the decomposition in 50% citric acid was proved by the observation that exclusion of air had no effect. Measurements in lower concentrations of citric acid were made without exclusion of air, but an appreciable proportion of the destruction was probably non-oxidative. Citrate reduces oxidation by forming complexes with heavy metal catalysts.

EFFECT OF FRUCTOSE AND RELATED SUBSTANCES

When it was found that anaerobic destruction in fruit and vegetable products was often greater than in pure buffer solution the effect of a number of carbohydrates, acids, and nitrogenous compounds, which occur naturally, was investigated. Most of the substances had no appreciable effect but more rapid destruction was promoted by fructose, sucrose, fructose 6 phosphate, and fructose 1.6 diphosphate. The effect of these substances on the velocity constant is given in Table 3.

TABLE 3

Effect of fructose and related substances on velocity constant

Temperature (°C.)	pH	Velocity constant in citrate-phosphate buffer with					
		No addition	Fructose 0.5M	Sucrose 0.5M	Fructose 6 phosphate 0.03M	Fructose 1.6 diphosphate	
						0.03M	0.05M
30	2.2	8.3×10^{-7}	8.8×10^{-7}	8.8×10^{-7}			—
	3.0	9.8	12.1	11.9			9.8×10^{-7}
	4.0	8.9	13.0	10.9			9.8
	5.0	3.6	8.4	3.6			10.6
	6.0	1.8	7.6	1.8			25.4
100	2.2	2.3×10^{-4}	5.2×10^{-4}	5.0×10^{-4}	2.9×10^{-4}	2.9×10^{-4}	
	3.0	3.9	10.5	10.5	—	4.6	
	4.0	5.8	16.5	14.4	9.6	9.6	
	5.0	2.9	19.3	8.9	9.6	14.4	
	6.0	1.9	21.0	5.2	14.4	23.1	

Solutions of fructose contain fructopyranose and fructofuranose in equilibrium with the former predominating. The proportion of fructofuranose has been shown by Gottschalk *(5)* to be about 12% at 0°C. and pH 4.3. From the effect of temperature on the specific rotation the proportion appears to be about 20% at 30°C. The accelerating effect of fructose appears to be due to the fructofuranose as the fructose phosphates, which are entirely furanose, have a much greater effect. At 30°C. and pH 4-5 the effect of 0.05M fructose diphosphate was similar to that of 0.5M fructose.

Glucose had no effect, and the effect of sucrose appeared to be due to fructose liberated by hydrolysis as it was similar to fructose at low pH and much less at higher pH. The effect of sorbose and hydroxymethylfurfural was similar to that of fructose at the same molar concentration and is probably negligible at the low concentrations in most fruit and vegetable products.

The accelerating effect of fructose and the fructose phosphates (as measured by the ratio of the velocity constants) increased with increasing pH. The relation is quite different from that of the reaction in pure buffer solution and suggests that the ascorbate ion plays the predominant role in the catalyzed reaction. The effect of fructose was much greater at 100°C. that at 30°C. At 100°C. the rate of the fructose-catalyzed reaction increased continuously with increasing pH while at 30°C. it followed the trend of the uncatalyzed reaction, reaching a maximum at pH 3-4. The effect of increasing temperature on fructose catalysis is probably due to the increasing proportion of fructofuranose (Isbell and Pigman, *10*).

The effect of sucrose was also greater at 100°C. It was equal to that of fructose up to pH 3 but very much less at higher pH. At 30°C. and pH 5-6 sucrose had no significant effect. Inversion is probably negligible under these conditions.

The times of half destruction are given in Table 4.

TABLE 4

Effect of fructose and related substances on time of half destruction

Temperature (°C.)	pH	Time of half destruction in citrate-phosphate buffer with					
		No addition	Fructose 0.5M	Sucrose 0.5M	Fructose 6 phosphate 0.03M	Fructose 1.6 diphosphate	
						0.03M	0.05M
		weeks	*weeks*	*weeks*			*weeks*
30	2.2	83	78	78			—
	3.0	70	57	58			70
	4.0	77	53	63			70
	5.0	190	82	190			65
	6.0	390	90	390			27
		hours	*hours*	*hours*	*hours*	*hours*	
100	2.2	50	22	23	40	40	
	3.0	30	11	11	—	25	
	4.0	20	7	8	12	12	
	5.0	40	6	13	12	8	
	6.0	60	5½	22	8	5	

RETENTION OF ASCORBIC ACID IN FRUIT AND VEGETABLE PRODUCTS

The results in ideal systems can now be related to data for retention of ascorbic acid after 18 months at 32°C. obtained by Brenner, Wodicka, and Dunlop *(1)* for a number of canned foods. These data are plotted in Fig. 1 together with pH-retention curves for the buffer solutions with and without fructose. The times of half destruction at 32°C. were obtained by interpolation and then converted to per cent retention after 18 months (78 weeks).

The ascorbic acid retention of orange juice was practically the same as that of the corresponding buffer solution, while the retention of apricots, spinach, lima beans, and peas was less. The concentration of fructose and sucrose in orange juice is apparently too low to affect the ascorbic acid retention, but the concentration should be much higher in the apricots which are normally canned in syrup. At the pH of the vegetables sucrose would be without effect, but low concentrations of fructose and fructose phosphates could have an appreciable effect.

The tomato juice retained ascorbic acid somewhat better than the corresponding buffer. However, this difference should not be regarded as evidence of a stabilizer as the analyses of tomato juice were not corrected for apparent ascorbic acid which tends to increase during storage. The effect of concentration does not support the presence of a stabilizer.

Concentrated products with a higher concentration of fructose and related substances would be expected to lose their ascorbic acid more rapidly, and this has been found to be the case. Curl *(2)* found that the loss of ascorbic acid from orange juice increased with concentration and Hum-

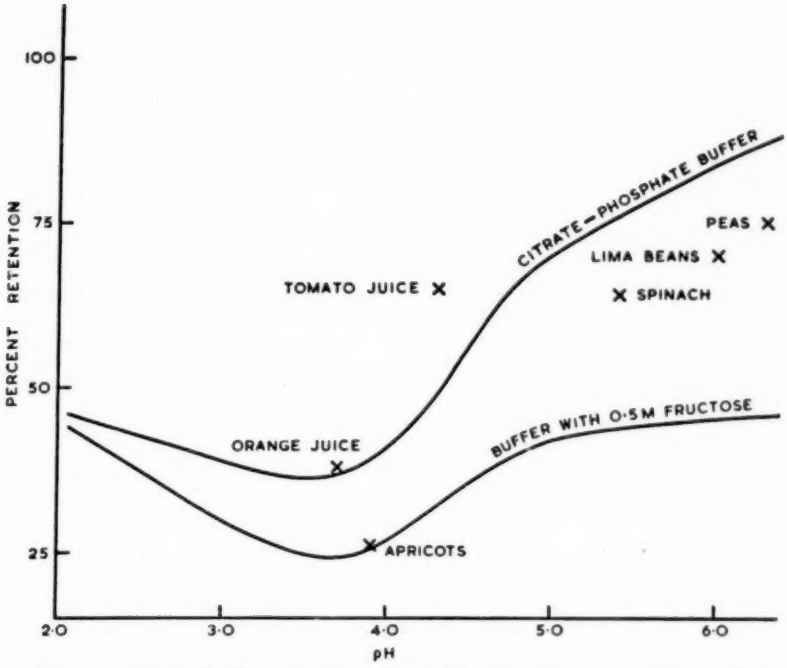

Figure 1. Effect of pH on retention of ascorbic acid after 18 months at 32°C. in buffer solutions and canned foods.

mel and Okey *(9)* obtained the same result with tomato juice. The author concentrated orange juice and aqueous extracts of cabbage and cauliflower *in vacuo* to an ascorbic acid level of 200 mg./100 ml. and studied the loss under anaerobic conditions at 100°C. The times of half destruction for orange, cabbage, and cauliflower concentrates were 5, 2½, and 5 hours, respectively, compared with 30, 46, and 54 hours for the corresponding buffers.

PRODUCTS OF DECOMPOSITION

In preliminary studies solutions of 0.25% ascorbic acid in distilled water were sealed in Florence flasks *in vacuo* and held at 100°C. for 10 days. During this period 93% of the ascorbic acid decomposed and the pH increased from 3.0 to 7.2. For each mole of ascorbic acid destroyed 0.43 mole of furfural and 1.05 mole of carbon dioxide were produced. The furfural was identified by color reaction with aniline-acetic acid, melting points of phenylhydrazone (99°C.) and dinitrophenylhydrazone (200°C.), and spectral absorption of dinitrophenylhydrazone in alkaline solution, and was determined as phloroglucide.

More detailed measurements were made at 30°C. in connection with the studies of the effect of pH and of fructose and related substances on ascorbic acid decomposition. The moles of furfural and carbon dioxide produced per mole of ascorbic acid decomposed after 104 weeks at 30°C.

are given in Table 5. The determinations of carbon dioxide were subject to a standard error of approximately 5 micromoles which involved large errors in the ratio for small losses of ascorbic acid.

The yield of furfural was determined primarily by pH, the added substances having relatively less effect. From nearly half a mole per mole of ascorbic acid at pH 2.2 it declined with increasing pH and became negligible at pH 5. To determine whether the differences could be due to subsequent destruction of furfural, solutions of pH 2.2-6.0 with a 0.005M concentration of furfural were held at 30°C. for 24 weeks, but no significant change occurred in this period.

The yield of carbon dioxide varied only slightly with pH and was generally reduced by the added substances. This conclusion is somewhat tentative in view of the comparatively large error of this determination.

Only in the pure buffer solution of pH 2.2 did the yields of furfural and carbon dioxide approximate to those obtained in the preliminary experiment at 100°C. It appears that the simple decomposition to these substances only predominates at high temperatures or acidities and other reactions become relatively more important with lowering of temperature or acidity.

From pH 2.2 to 4.0 the loss of ascorbic acid varied less than the production of furfural or carbon dioxide. A plausible interpretation is that the production of these substances does not occur in the primary reaction but is determined by the relative rate of various secondary reactions.

TABLE 5

Production of furfural and carbon dioxide from ascorbic acid at 30°C.

pH	Added substance	Micromoles in 25 ml.			Moles per mole of ascorbic acid	
		Ascorbic acid decomposed	Furfural produced	Carbon dioxide produced	Furfural	Carbon dioxide
2.2	Nil	138	66	143	0.48	1.04±0.04
	Fructose	147	61	59	0.41	0.40±0.03
	Sucrose	147	63	85	0.43	0.58±0.03
3.0	Nil	153	26		0.17	
	Fructose	176	23		0.13	
	Sucrose	176	23		0.13	
	Fructose diphosphate	155	36		0.23	
4.0	Nil	145	4	95	0.03	0.66±0.03
	Fructose	188	3	115	0.02	0.61±0.03
	Sucrose	171	2	113	0.01	0.66±0.03
	Fructose diphosphate	161	4	—	0.02	—
5.0	Nil	70	0		0	
	Fructose	149	0		0	
	Sucrose	79	0		0	
	Fructose diphosphate	172	0		0	
6.0	Nil	34	0	24	0	0.70±0.15
	Fructose	141	0	66	0	0.47±0.04
	Sucrose	36	0	21	0	0.58±0.14
	Fructose diphosphate	240	0	68	0	0.28±0.02

(Fructose and sucrose, 0.5M; fructose diphosphate, 0.05M)

SUMMARY

The anaerobic decomposition of ascorbic acid was studied at 30°C. and 100°C. from pH 2.2 to 6.0.

In citrate-phosphate buffer the reaction proceeded most rapidly at pH 3-4. It was accelerated by fructose, fructose 6 phosphate, and fructose 1.6 diphosphate, the effect increasing with pH. The effect of sucrose appeared to be due to liberation of fructose, as it was equal to that of fructose up to pH 3 and much less at higher pH.

The results are considered in relation to the retention of ascorbic acid by fruit and vegetable products.

Furfural and carbon dioxide were the main products of decomposition at high temperatures or acidities. With lowering of temperature or acidity other products become important.

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LITERATURE CITED

1. Brenner, S., Wodicka, V. O., and Dunlop, S. G. Effect of high temperature storage on the retention of nutrients in canned foods. *Food Technol.*, **2**, 207 (1948).
2. Curl, A. L. Concentrated orange juice storage studies. The effects of degree of concentration and temperature of storage. *Canner*, **105**, No. 13, 14 (1947).
3. Duncan, I. J. Determination of furfural. *Ind. Eng. Chem., Anal. Ed.*, **15**, 162 (1943).
4. Feaster, J. F., Tompkins, M. D., and Pearce, W. E. Effect of storage on vitamins and quality in stored canned foods. *Food Research*, **14**, 25 (1949).
5. Gottschalk, A. The proportion of fructo-furanose present in d-fructose solution at equilibrium (0°C.). *Austral. J. Exp. Biol.*, **21**, 139 (1943).
6. Herbert, R. W., Hirst, E. L., Percival, E. G. V., Reynolds, R. J. W., and Smith, F. Constitution of ascorbic acid. *J. Chem. Soc.* **1933**, 1270.
7. Horner, G. Gases in canned foods. Ann. Rep. Fruit and Veg. Pres. Sta. Campden, **1933-4**, 50 and **1934-5**, 44.
8. Huelin, F. E., and Stephens, I. M. The copper-catalysed oxidation of ascorbic acid in fruit and vegetable suspensions. *Austral. J. Sci. Res.*, **B1**, 50 (1948).
9. Hummel, M., and Okey, R. Relation of concentration of canned tomato products to storage losses of ascorbic acid. *Food Research*, **15**, 405 (1950).
10. Isbell, H. S., and Pigman, W. W. Pyranose-furanose interconversions with reference to the mutarotations of galactose, levulose, lactulose, and turanose. *J. Res. Natl. Bur. Standards*, **20**, 773 (1938).
11. Lamden, M. P., and Harris, R. S. Browning of ascorbic acid in pure solutions. *Food Research*, **15**, 79 (1950).
12. Wokes, F., Organ, J. G., and Jacoby, F. C. The estimation of apparent vitamin C in foods. *J. Soc. Chem. Ind.*, **62**, 232 (1943).

EFFECT OF SPROUTING AND COOKING OF SOYBEANS ON PALATABILITY, LYSINE, TRYPTOPHANE, THIAMINE, AND ASCORBIC ACID

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The value of the proteins of soybeans has been demonstrated in the successful growth of rats on rations in which soybeans were the sole source of protein *(11, 15, 22)*. There is considerable evidence also that the human species can satisfactorily digest and utilize soybean proteins *(7, 19, 24)*. Recent work has shown that soybeans are a good source of vitamins as well as an excellent source of protein *(7, 9, 15, 20, 24)*.

Attention has been turned to the mobilization or synthesis of the nutrients during sprouting. Several reports concerning the nutritive value of sprouting soybeans have been found *(1, 14, 33)* but there are few reports on the effect of cooking on sprouted soybeans *(9, 16)*. The mobilization of the nutrients during sprouting may make any effect of cooking much more apparent than would be the case with dry soybeans. Therefore the cooking of sprouted soybeans was of interest.

The purpose of this study was to determine the effects of sprouting and of 3 methods of cooking on the palatability of the sprouted soybeans and their lysine, tryptophane, thiamine, and ascorbic acid contents. How the nutrients mentioned above were distributed between the seeds and sprouts of the sprouted beans was also determined. Cooking methods were (I) boiling in unsalted water, (II) boiling in salted water, and (III) sautéing in a small amount of salted water and lard, which is a Chinese method. As used in this paper the term "sprouted beans" refers to the bean and sprout.

EXPERIMENTAL

Soybeans. Beans of the Seneca variety, harvested in 1949, were obtained from the Cornell University Farm in January, 1950. During progress of the study the dry beans were kept in covered glass jars in a "walk-in" refrigerator at 40°F.

The seeds were carefully hand picked and all but clean whole beans were discarded. The beans were washed with twice their volume of tap water, and then soaked in 10% Clorox (active ingredient sodium hypochlorite) for 3 minutes. Because of stickiness of the seed coat after Clorox treatment, the soaked beans were washed 5 times as before. Treated beans in 400-g. lots were soaked in 1200 ml., 15°C. tap water for 10 hours. In the study of distribution of nutrients, 700 g. of beans were soaked in 2100 g. of water. After soaking, the beans were spread on a perforated plate set in a glass cylinder 18 in. high and 8 in. in diameter with a hole at the lower side. The top of the cylinder was covered with a piece of wet cheese-cloth to prevent excess ventilation which is unfavorable to sprouting *(3, 9)* and the room was darkened to prevent the developing of green color due to chlorophyll formation. The beans were sprinkled with tap water at 12 to 15°C. for 1 min. every 4 hours during the day by means of a household spray. Thus the swollen and sprouted seeds were periodically rinsed so that bacteria or molds, which might have developed, were carried away. During the night the beans were left covered with wet cheese-cloth, and the next morning sprinkling

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was continued. The beans were sprouted for from 6 to 7 days before cooking, depending upon the room temperature which ranged from 17 to 27°C. during this study. Wilson *(31)* stated that within the range of 10 to 30°C., the higher the temperature the faster the rate of germination.

Sampling. *Dry Soybeans.* Dry beans in 300-g. lots were ground in a model No. 1 Wiley Mill with a sieve having meshes of 1/32 in. diameter and with a knife of 4 blades (3 in. across, overall depth of 1¾ in., thickness ⅜ in.). All samples were taken in duplicate: 15 g. for analysis of amino acids, 50 g. for thiamine, 10 g. for ascorbic acid, and 5 g. for moisture.

Sprouted Beans. At the end of sprouting, the beans were rinsed with tap water and rootlets and poor beans were removed by hand. The edible portion ranged from 62 to 87%.

In nutrient distribution studies, one lot of 1000 g. was separated into beans and sprouts. Beans and sprouts were chopped separately on a board with a knife having a sharp chromium blade, mixed quickly, and sampled in duplicate. In another lot of 500 g., sprouted soybeans were chopped together and the samples taken for analysis.

Cooked Sprouted Beans. Cooked sprouted beans were drained in an enamel colander for one-half minute. They were sampled for palatability, and approximately 1 hr. later samples were taken for chemical analysis. Samples of the uncooked bean sprouts were also taken just prior to each cooking.

For determinations of the distribution of amino acids, 25 g. of chopped sprouts, of chopped beans, and of chopped sprouted beans were covered with 10% HCl for lysine analysis, and with 5 N NaOH for tryptophane determination. Five-g. samples were taken at random from the raw and cooked lots, and treated as above. Samples were stored in a refrigerator for approximately 24 hrs. until they could be ground and autoclaved.

For thiamine analysis in the distribution study, 75 g. of chopped sprouts, of chopped beans, and of chopped sprouted beans were covered with 0.1 N H_2SO_4 and kept in the refrigerator overnight before analysis could be completed. To study the effect of cooking, 25-g. random aliquots were taken from each raw and cooked lot and treated in the same manner.

Ten-g. samples of each raw and cooked lot, after one hour of holding, were taken for ascorbic acid determinations and immediately covered with 3% H_3PO_3. Samples were kept in a covered beaker in the refrigerator, 40°F., for approximately 5 hrs. before analysis could be made.

Moisture was determined on duplicate 10-g. samples of sprouts, beans, and sprouted beans.

Chemical methods. Lysine was determined by microbiological assay with *Leuconostoc mesenteroides* P-60 on a medium similar to that described by Stokes *et al. (28)*, Brin *(6)*, and Dunn *(12)* except that 3 of the amino acids—norleucine, norvaline, and hydroxyproline—were omitted as suggested by Steele *et al. (27)*.

Tryptophane. The medium of Wooley and Sebrell *(32)* and microorganism *L. arabinosus 8014* were used for microbiological analysis of tryptophane.

Thiamine. Thiamine was determined by the modified thiochrome method of Connor and Straub *(10)* as adapted for vegetables by Moyer and Tressler *(17)*. The extracts were stored in covered glass bottles at 0°F. until analysis could be completed.

Ascorbic Acid. Ascorbic acid was determined by the xylene method of Nelson and Somers *(18)*.

Moisture. The samples were dried at 99-100°C., 100 mm. Hg. vacuum (A.O.A.C. method, 2).

Cooking methods. *Boiled in Unsalted Water.* 275 g. (4 servings) of sprouted beans were added to 200 g. of rapidly boiling tap water in a 2-quart covered enamel saucepan. The pan was covered and the beans simmered 20 minutes. The water returned to the boil within 1½ minutes after the sprouts were added. Rate of evaporation was controlled in each study by the use of a gas burner and manometer arrangement.

Boiled in Salted Water. 2.6 g. of salt and 275 g. of sprouted beans were added to 200 g. boiling water. Cooking method was the same as above except simmering was 2 minutes less.

Sautéed. 120 g. of boiling water, 6 g. of salt, and 12 g. of lard were placed in a covered A-G 833 B model pyrex saucepan with a capacity of 1½ quarts; 275 g. of

sprouted beans were added; and 1½ minutes were required for the water to return to the boil and 12 minutes for simmering.

In each study the sprouted beans were stirred once thoroughly with a silver plated fork at the mid-point of simmering. After cooking, the sprouted beans were drained for one-half minute.

Palatability. Palatability tests were run immediately after cooking. Training of the 5 judges to be familiar with the characteristics of this Oriental vegetable, particularly the nut-like texture, proved very important. The untrained judges expected the beans to be mealy which they are not, even though they are cooked for long periods. The judges were asked to score beans and sprouts separately as well as combined. Each of the quality factors was scored on the basis of ten points. Ten was equivalent to *extremely good;* 8, *good;* 6, *average;* 4, *fair;* and 2, *very poor.*

Statistical treatment. Percentage retention of amino acids and vitamins in sprouted beans as served was calculated on total weights of raw and cooked sprouted beans in each cooking study. Standard errors were calculated and used in ascertaining by Student's "t" method *(26)* significance at the 5% level of the difference of the observed retention of each amino acid and vitamin in each cooking study.

RESULTS AND DISCUSSION

Treatment of the beans with Clorox before sprouting prevented mottling of the beans and resulted in a golden, succulent product, which in China is more acceptable than are the green-colored ones. It also retarded growth of microorganisms.

Palatability. Differences in palatability were not statistically significant (Table 1). Average palatability scores were: boiled in unsalted water 6.5, boiled in salted water 6.7, sautéed 7.0.

Amino acids. *Lysine.* The lysine content in dry soybeans was found to be 1767 mg. per 100 g. while Block and Bolling *(4)* reported 1810 mg. per 100 g. Sprouting decreased the lysine retention in the edible portion to 36% (Table 2). This agreed with findings of several workers *(21, 23)* that α-amino acids and peptones are decomposed in germinating seeds by the splitting of free α-amino groups in the form of ammonia.

The content of lysine on the moist basis was 88 mg. per 100 g. of sprouts and 358 mg. per 100 g. of beans (Table 3). The sprouts contained 5.4% and the beans 34.0% of the original lysine content in the dry beans. The content was slightly higher in the sprouts than in the beans when calculated on the dry basis; namely, 1517 and 1321 mg. per 100 g.

TABLE 1

The effect of three methods of cooking on palatability

Cooking method	Palatability score[b]										Total
	Appearance		Color		Aroma	Taste		Tenderness			
	Sprouts	Beans	Sprouts	Beans	Sprouts and beans	Sprouts	Beans	Sprouts	Beans	Sprouts and beans	
Boiled in unsalted water	6.4	6.1	6.3	6.1	7.5	6.6	6.9	6.4	6.6	6.5	6.5
Boiled in salted water	6.6	6.1	6.2	6.4	7.1	6.9	7.4	6.6	7.2	6.9	6.7
Sautéed	6.5	6.9	6.8	6.6	7.5	7.6	7.8	6.2	6.8	7.1	7.0

[b]Average score of 5 judges for 5 times. Each factor was scored on the basis of ten points: 10 equivalent to *extremely good;* 8, *good;* 6, *average;* 4, *fair;* and 2, *very poor.*

TABLE 2

The effect of sprouting on amino acids and vitamins of soybeans [c]

Nutrient	Content				Retention after sprouting	
	Moist basis		Dry basis			
	Dry soybeans (before sprouting)	Sprouted soybeans (after sprouting) (E.P.)[d]	Dry soybeans	Sprouted soybeans (E.P.)[d]	A.P.[e]	E.P.[d]
	mg./100 g.	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	%	%
Lysine	1767.0	238.0	2010.0	1384.0	39	36
Tryptophane	454.0	104.0	516.0	604.1	66	61
Thiamine	1.0	0.2	1.1	1.1	58	48
Ascorbic acid	3.6	18.0	4.1	104.7	145	132

[c] The data are averages of 5 replicates.
[d] From the 400 g. of dry soybeans used in each replicate the average weight of sprouted soybeans obtained was 1448 g. The edible portion ranged from 62 to 87% with an average of 73%. These are the data used in calculating % retention.
[e] A.P. refers to the lot of sprouted beans before the poor ones were removed. E.P. refers to the sprouted beans remaining.

No statistically significant differences in the retention of lysine were found among the 3 cooking methods. The percentage retentions were 92 ± 2.0 when cooked in unsalted water, 93 ± 2.1 in salted water, and 89 ± 4.1 in sautéed (Table 4).

Tryptophane. Tryptophane content in dry soybeans, 456 mg. per 100 g., was also lower than that reported by Block and Bolling, 535 mg. per 100 g. *(4)*. Sprouted beans retained 61% on their tryptophane (Table 2). Cooking effect was greater on the tryptophane than on the lysine content in this study. Whether the indole group in the tryptophane molecule made it more labile to heat or whether tryptophane was more soluble than lysine was not known. No statistically significant differences in tryptophane retention was noted among the three methods of cooking, 77 ± 4.1% to 85 ± 2.2% (Table 4).

Vitamins. *Thiamine.* Dry soybeans contained 1.1 mg. of thiamine per 100 g. on the moist basis (Table 2). Retention after sprouting in the edible portion was 48%. This agreed with reports in the literature *(8, 13, 29)*.

No statistically significant differences in retentions were found among the 3 methods of cooking, 63 ± 3.9% to 72 ± 3.4% (Table 4).

Ascorbic Acid. The dry beans contained 4.2 mg. of ascorbic acid per 100 g. on the moist basis, and 4.8 mg. on the dry basis (Table 3). The uncooked sprouts on the moist basis contained about one-half as much ascorbic acid, 10.6 mg., as the beans, 20.6 mg. per 100 g. On the dry basis, however, the sprouts contained twice as much, namely 182.8 mg. in the sprouts and 76.0 mg. per 100 g. in the beans.

No statistically significant differences in ascorbic acid retention occurred among the three methods of cooking and holding of the cooked sprouted beans for one hour, 27 ± 3.3% to 38 ± 1.9%.

Moisture. Dry beans contained 12% moisture, sprouts 94, beans 73, and sprouted beans 83% (Table 3).

SUMMARY

Retentions of 2 amino acids and 2 vitamins during sprouting, and during cooking of sprouted beans by 3 methods were investigated and

TABLE 3

The content of amino acids and vitamins in sprouts and beans

Botanical part	Weight of sample used	Moisture	Content on moist basis[f]				Percentage of original content in the botanical parts[f]			
			Lysine	Trypto-phane	Thiamine	Asco bic acid	Lysine	T ypto-phane	Thiamine	Ascorbic acid
	g.	%	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*	%	%	%	%
Dry beans	700	12.1	1837	456	1.10	4.2	100	100	100	100
Sprouted beans	2028	82.8	248	108	0.25	16.4	39	61	66	1131
Sprouts	796	94.2	88	58	0.08	10.6	5.4	5.5	8.3	287
Beans	1232	72.9	358	212	0.35	20.6	34	50	56	863

[f] Data are averages of 2 replicates. The proportion of sprouts to beans in the sprouted beans varied from sample to sample.

TABLE 4

The effect of three methods of cooking and holding[g] on the retention[h] of amino acids and vitamins in sprouted soybeans

Cooking method	Cooking time		Weight				Lysine			T yptophane			Thiamine			Ascorbic acid		
			Vegetable		Water		Content		Retention ±S.E.[i]	Content		Retention ±S.E.[i]	Content		Retention ±S.E.[i]	Content		Retention ±S.E.[i]
	To reach boiling	Simmer-ing	Raw	Cooked	Before cooking	After cooking	Raw	Drained vegetable	Drained vegetable	Raw	Drained vegetable	Drained vegetable	Raw	Drained vegetable	Drained vegetable	Raw	Drained vegetable	Drained vegetable
	min.	*min.*	*g.*	*g.*	*g.*	*g.*	*mg./100 g.*	*mg./100 g.*	%	*mg./100 g.*	*mg./100 g.*	%	*mg./100 g.*	*mg./100 g.*	%	*mg./100 g.*	*mg./100 g.*	%
Boiled in unsalted water	1½	20	275	245	200	167	238	245	92±2.0	104	90	78±3.4	0.180	0.146	72±3.4	18.0	5.3	27±3.3
Boiled in salted water	1½	18	275	244	200	186	238	246	93±2.1	104	90	77±4.1	0.180	0.128	63±3.9	18.0	5.8	32±1.7
Sautéed	1½	12	275	235	120	136	238	251	89±4.1	104	102	85±2.2	0.180	0.143	68±8.0	18.0	7.3	38±1.9

[g] Held in open pan at room temperature for approximately one hour. [h] Data are averages of 4 replicates. [i] Percentage retentions were calculated on total weight of vegetable at beginning and at end of cooking. No statistically significant differences were noted among these 3 cooking methods at 5% level.

recorded. Distribution of nutrients and moisture in the beans and sprouts were also determined.

LITERATURE CITED

1. Adolph, W. H., and Wu, C. M. Additional notes on soybean products. *Natl. Med. J.*, **6**, 231-233 (1920).
2. Assoc. of Official Agricultural Chemists. *Official and Tentative Methods of Analysis*. 6th ed., 1945, Washington, D. C.
3. Beeskow, H. C. Bean sprouts: Their preparations and properties. Mich. State College Agr. Expt. Sta. *Tech. Bull. No. 184* (1944).
4. Block, R. J., and Bolling, D. *The Amino Acid Content of Proteins and Foods*. 1945, Charles C. Thomas, Springfield, Ill.
5. Bricker, M., Mitchell, H. H., and Kinsman, G. M. The protein requirements of adult human subjects in terms of the protein contained in individual foods and food combination. *J. Nutr.*, **30**, 269 (1945).
6. Brin, M. The evaluation of some lactic acid producing bacteria in the microbiological assay. Unpublished M. S. Special Problem, School of Nutrition, Cornell University, 1948.
7. Burkholder, P. R. Vitamins in edible soybeans. *Science*, **98**, 188-190 (1943).
8. Burkholder, P. R., and McVeigh, J. The increase of B vitamins in germinating seeds. *Proc. Natl. Acad. Sci.*, **28**, 440 (1942).
9. Cheng, Stella Y. Y. The effect of sprouting on the nutritive value of soybeans: The ascorbic acid content and the protein quality. (Unpublished Ph.D. thesis, Cornell University, 1944.)
10. Connor, R. T., and Straub, G. J. Determination of thiamine by the thiochrome reaction. *Ind. Eng. Chem., Anal. Ed.*, **13**, 380-384 (1941).
11. Daniels, A. L., and Nichols, N. B. The nutritive value of the soybeans. *J. Biol. Chem.*, **32**, 91-102 (1917).
12. Dunn, M. S. Determination of amino acids by microbiological assay. *Physiological Rev.* **29**, 219 (1949).
13. French, C. E., Berryman, G. H., Goorley, J. T., Harper, H. A., Harkness, D. M., and Thacker, E. J. The production of vitamins in germinated peas, soybeans and other beans. *J. Nutr.*, **28**, 63-70 (1944).
14. Gallup, W. D., and Reder, R. E. Sprouted cowpeas as a source of protein and vitamins. *Proc. Oklahoma Academy of Science* **24**, 53 (1944).
15. Hayward, J. W. The nutritive value of soybean oil meal prepared by the different methods of oil extraction. *Oil and Soap*, **14**, 317 (1937).
16. Lee, F. A., and Whitcomb, J. Effect of freezing preservation and cooking on vitamin content of green soybeans and soybean sprouts. *J. Am. Diet. Assoc.*, **21**, 696 (1945).
17. Moyer, J. C., and Tressler, D. K. Determination of thiamine in vegetables. *Ind. Eng. Chem., Anal. Ed.*, **14**, 788 (1942).
18. Nelson, W. L., and Somers, G. G. Determination of ascorbic acid: Application of the indophenol-xylene extraction method to determination in large numbers of tomato juice samples. *Ind. Eng. Chem.*, **17**, 754 (1945).
19. Neumann, R. O. Die Sojabohnen und ihre verwertung im organismus. *Arch. für Hygiene*, **99**, 1 (1928).
20. Norris, L. C., Wilgus, H. S. Jr., Ringrose, A. T., Heinman, V., and Heuser, G. F. The vitamin G requirement of poultry. Cornell Expt. Sta. *Bull. 660* (1936).
21. Oparin, A. Catabolism of proteins in germinating seed. *J. Russian Botan. Congress* **1**, 22 (1921).
22. Osborne, T. B., and Mendel, L. B. The use of soybean as food. *J. Biol. Chem.*, **32**, 369-376 (1917).
23. Prisanischnikow, cited by Oslow in: *Principles of Plant Biochemistry*, 1931.
24. Scherinert, A., and Schieblich, M. Vitamin content of fresh soybeans. *Biedermanns Zeutr. B. Treiernähr*, **7**, 198-204 (1935).
25. Smith, A. H. (Personal communication, Jones, D. B., cited in *J. Am. Chem. Soc.*, **60**, 823, 1938.)
26. Snedecor, G. W. Statistical Methods 1946, Collegiate Press Inc., Ames, Iowa.
27. Steele, B. F., Sauberlich, H. E., Reynolds, M. S., and Baumann, C. A. Media for *Leuconostoc mesenteroides P-60* and *Leuconostoc citrovorum 8081*. *J. Biol. Chem.*, **157**, 141 (1945).
28. Stokes, J. L., Gunness, M., Dwyer, I. M., and Caswell, M. C. Microbiological method for the determination of amino acids. II. A uniform assay for the ten essential amino acids. *J. Biol. Chem.*, **160**, 35 (1945).
29. Wai, K. N. T., Bishop, J. C., Mack, P. E., and Cotton, R. H. The vitamin content of soybeans and soybean sprouts as a function of germination time. *Plant Physiol.*, **22**, 117 (1947).
30. Werner, G. Jaffe. The comparative biological value of legumes of importance in the Venezuelan diet. *Arch. Venezolanos Nutrition*, **1**, 107 (1950).
31. Wilson, H. K. Wheat, soybean and oats germination studies with particular reference to temperature relationship. *J. Am. Soc. Agron.*, **20**, 599-619 (1928).
32. Woolley, J. G., and Sebrell, W. H. Two microbiological methods for the determination of l(−)-Tryptophane in proteins and other complex substances. *J. Biol. Chem.*, **157**, 141 (1945).
33. Wu, H. Nutritive value of Chinese foods. *Chinese J. Physiol., Rept.*, Series **1**, 153-186 (1928).

THE NUTRITIVE REQUIREMENTS OF ACETOBACTER. II. THE VITAMIN SYNTHESIS OF *ACETOBACTER XYLINUM*[a]

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Acetic acid in the form of vinegar has been known for thousands of years. Its production on a world-wide basis is tremendous. In the United States alone, for example, more than 150,000 tons of acetic acid and its derivatives were produced in a single year (1941). Yet, the commercial process by which ethanol is converted to acetic acid is still considered more an art than a science.

Although the chemistry of this reaction and the mechanisms of the acetification process are thought to be understood, little is known concerning the nutritive requirements of the bacteria that bring about the reaction. The supporting medium for these bacteria has much to do with the duration and the success of the acetic acid fermentation, but only a comparatively small amount of research has been reported concerning the factors governing the growth of these organisms. Very little information can be found regarding the requirements of vitamins, amino acids, and organic and inorganic compounds vital to this conversion. The same holds true for the environmental factors such as oxygen, pH, alcohol and acid concentrations, and metabolites produced.

Underkofler, Bantz, and Peterson *(4)* have reported that *Acetobacter suboxydans* requires p-aminobenzoic acid (PABA), pantothenic acid, biotin, and nicotinic acid. Foda and Vaughn *(2)* recently reported that *A. melanogenum*, *A. oxydans*, and *A. rancens* required PABA, pantothenic and nicotinic acids. *A. melanogenum* and *A. rancens* were found to have an additional requirement for thiamine. Litsky, Esselen, Tepper, and Miller *(3)* in a study of the nutritional requirements of *A. xylinum*, found that PABA was the only one of the B vitamins needed for growth. Since previous studies of the various species of *Acetobacter* indicated that from 4 to 5 vitamins were necessary for growth, the following investigation was proposed to ascertain whether these vitamins are vital to the metabolism of *A. xylinum*—and, if so, to determine how much of the vitamins are synthesized by this organism.

EXPERIMENTAL

Organism and inoculum. The culture of *A. xylinum*, obtained from the National Institute of Health, was carried on slants of yeast extract agar.

Inocula were obtained from 24-hour slant cultures that had been transferred daily for at least 3 days. The cells were removed from the agar slant with 10 ml. of sterile physiological saline (0.85%) and were washed 3 times. The washed cells were finally suspended in 10 ml. of saline. One-tenth ml. of this suspension was used to inoculate

[a]Contribution No. 897 from the Massachusetts Agricultural Experiment Station, Department of Bacteriology and Public Health, University of Massachusetts, Amherst, Massachusetts.

10 ml. of the test medium in a 125-ml. Erlenmeyer flask. The inoculated flasks were incubated at 30°C. for 48 hours, and were shaken continuously in a shaking machine to provide aeration throughout the incubation period.

Media. The basal medium was the same as that employed by Litsky *et al. (3)*. It contained per 100 ml.: Difco vitamin-free casamino acids, 0.5 g.; dextrose, 3.0 g.; adenine sulfate, 0.002 g.; guanine hydrochloride, 0.002 g.; uracil, 0.002 g.; K_2HPO_4, 0.05 g.; KH_2PO_4, 0.05 g.; $MgSO_4 \cdot 7\ H_2O$, 0.02 g.; NaCl, 0.001 g.; $FeSO_4 \cdot 7\ H_2O$, 0.01 g.; and $MnSO_4 \cdot 3\ H_2O$, 0.001 g. PABA was added in the concentration of 20 micrograms per 100 ml. of medium. The medium was adjusted to pH 6.0 and sterilized at 121°C. for 15 minutes.

Transfer to test medium. Because of the nature of the slimy film growth of *A. xylinum*, it was difficult to wash the cells of the inoculum to remove all traces of growth factors carried over from the yeast extract agar. To reduce the effect of these carry-over substances to a minimum, the organism was passed through several flasks of test medium. The fourth series was usually employed to test for vitamin synthesis.

Vitamin assay. After 48 hours of incubation, several of the cultures of the fourth transfer series were pooled and filtered through a Chamberlain-Pasteur filter to remove the bacteria. The clear filtrate was then assayed for the following vitamins: pantothenic acid, by the method of Skeggs and Wright *(1)*, employing *Lactobacillus arabinosus* 17-5; biotin, by the method of Skeggs and Wright *(1)*, employing *L. arabinosus* 17-5; niacin, by the method of Snell and Wright *(1)*, employing *L. arabinosus* 17-5; pyridoxine, by the method of Atkins *et al. (1)*, employing *Saccharomyces carlsbergensis;* riboflavin, by the fluorometric method; thiamine, by the thiochrome method. Filtered medium, both uninoculated and inoculated, and not incubated, served as controls to determine whether vitamin contamination occurred from the medium, the filter, or the inoculum. The results of this study are summarized in Table 1.

TABLE 1

The vitamin analysis of the filtered media (Millimicrograms of vitamins per ml.)

Vitamins	Basal	Seeded basal before incubation	Seeded basal after incubation
Pantothenic acid	0	0	6.6
Biotin	0	0	0.45
Niacin	0	0	1.1
Pyridoxine	0	0	36.0
Riboflavin	0	0	34.0
Thiamine	0	0	0

RESULTS AND DISCUSSION

From the results of the controls used in this investigation and summarized in Table 1, it is evident that the basal medium in which *A. xylinum* was grown, was free from the vitamins in question before inoculation. It is also evident that the filter, used to clarify the test medium, did not contaminate the uninoculated medium when filtered. To determine whether significant vitamin carry-over occurred during the seeding of the flasks from the third to the fourth transfer, the inoculated medium was assayed prior to incubation. No measurable vitamin carry-over was indicated. The basal medium, in which *A. xylinum* was grown for 48 hours, contained most of the B vitamins in amounts that were easily detectable. The fact that thiamine was the only vitamin not detected in the medium after incubation suggests that, if thiamine is synthesized by *A. xylinum*, the amounts produced are not in excess of its needs.

A. xylinum was found to produce pantothenic acid, biotin, niacin, pyridoxine, and riboflavin in amounts that were very easily detectable in the medium supporting growth. Other species of *Acetobacter* require some of these vitamins for growth *(2, 4)*. It is highly probable that *A. xylinum* may synthesize the above vitamins in amounts that may help to support the growth of the other acetic acid bacteria.

In the commercial manufacture of vinegar, an uncontrolled mixed culture of organisms is used, and each new batch is seeded from a previous batch. Consequently, the possibility of commensalism among the organisms in the culture must be considered. That is, some of the component organisms may not be able to produce all the vitamins they require; but the whole culture collectively may take care of any deficiencies. The results of this investigation indicate such a possibility.

At the present time there is a controversy regarding the usefulness of "mother of vinegar" in the generator. Because of its type of growth, mother of vinegar is thought to be predominantly *A. xylinum* by some investigators. If this be the case, mother of vinegar could have a definite purpose and use in the generator, that of supplementing the vitamins required for the growth of the more rapid acetic acid producing bacteria.

SUMMARY

Of the B vitamins investigated, it was found that pantothenic acid, biotin, pyridoxine, niacin, and riboflavin were synthesized by *A. xylinum*. The significance of the vitamin synthesis is discussed.

LITERATURE CITED

1. Association of Vitamin Chemists, Inc. *Methods of Vitamin Assay*. 2nd ed., 1951, Interscience Publishers, Inc., New York, N. Y.
2. Foda, I. O., and Vaughn, R. H. The nutritional requirements of *Acetobacter melanogenum* and related species. *J. Bact.*, **65**, 79 (1953).
3. Litsky, W., Esselen, W. B., Jr., Tepper, B. S., and Miller, G. The nutritive requirements of Acetobacter. I. Vitamin requirements of *Acetobacter xylinum*. *Food Research*, **18**, 250 (1953).
4. Underkofler, L. A., Bantz, A. C., and Peterson, W. H. Growth factors for bacteria. XIV. Growth requirements of *Acetobacter suboxydans*. *J. Bact.*, **45**, 183 (1943).

THE EFFECT OF HOMOGENIZED MILK UPON THE VISCOSITY OF CORNSTARCH PUDDINGS[a,b]

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(Manuscript received June 3, 1953)

With the wider distribution of homogenized milk for home and institution use, problems have arisen concerning its behavior in cookery. Investigations have been reported upon its use in escalloped potatoes and cooked cereals *(9)*, in custards *(5, 9)*, and in white sauces *(19)*. No reports were found on its use in cornstarch puddings, although in their paper on white sauces, Towson and Trout *(19)* commented briefly that cornstarch puddings made with homogenized milk were smoother and more viscous than similar ones made with nonhomogenized milk. Preliminary work *(17)* by one of the authors indicated that cornstarch puddings made with homogenized milk were undesirably more firm in consistency than puddings made with nonhomogenized milk for which the same standard formula was used.

The purpose of the study here reported was twofold: (a) to determine the reduction in the amount of cornstarch in the pudding formula that would yield a pudding made with homogenized milk similar in consistency to that of the nonhomogenized milk pudding and (b) to seek a possible explanation for the difference noted in the consistency of the puddings made from the two kinds of milk. The report consists of two parts based upon these objectives. Two series of puddings were made in Part I and three sets in Part II.

PART I

EXPERIMENTAL PROCEDURE

Materials and equipment. Pasteurized whole milk and milk from the same lot homogenized under 2,500 pounds' pressure were obtained as needed from the Purdue University Creamery. The same brand of commercial cornstarch was used throughout the study, and for any one series of puddings, a sufficient amount for all the puddings was secured, mixed, and stored in a closely covered metal container until weighed out for use. The granulated sugar and table quality salt were the same throughout the investigation.

For cooking the puddings, the upper part of a double boiler (¾ quart capacity) was used in a water bath, size 14″ × 14″ × 5″, which required 15 quarts of water to fill it to the desired level.

The pH of the milks was determined by means of a Beckman Model G pH Meter. In the first part of the study, the relative consistency of the puddings was measured with a Precision Penetrometer (Food Model No. 3512) with an aluminum cone (No. 3527). For the later experiments there was also available a Brookfield Synchro-Lectric Viscometer, Model RVF, for measuring the apparent viscosity of the puddings.

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[b]A portion of the data included in this paper is from a thesis written by Elizabeth Shaw in partial fulfillment of the requirements for the degree of Master of Science in Home Economics at Purdue University, January 1952.

Formula. The basic formula was selected after numerous recipes for plain cornstarch puddings found in household cookbooks had been studied and many of them tried in preliminary tests. The proportions of ingredients adopted as the basic formula were:

Ingredients	Measure	Weight in grams
Cornstarch	2 tablespoons	18
Sugar	4 tablespoons	50
Salt	⅛ teaspoon	1
Milk	2 cups	488

The proportion of starch to milk in this formula is 3.69 g. cornstarch per 100 g. milk.

Method of cooking. It is known that small variations in rate and temperature of cooking and in manipulation may have a marked effect upon the consistency of starch pastes *(1, 2, 4, 7, 15)*. Hence, in any study involving the use of starch as a thickening agent, careful consideration must be given to every small detail of the procedure in preparing and handling the cooked product.

After considerable experimentation *(17)* the following method of cooking was found to give reproducible results. The water in the water bath was adjusted to the desired level at the beginning of the cooking period for each pudding and was kept at an active boil throughout that period. The dry ingredients were weighed into a 400-ml. beaker and thoroughly mixed. The cold milk was weighed into the upper part of a double boiler; a portion of this milk (80 ml.) was reserved and blended with the dry ingredients. The remainder of the milk was heated to 90°C. over boiling water, added to the cornstarch mixture and blended with 20 strokes. The initial temperature of this mixture was noted before it was poured into a double boiler top which was preheated and in place in the water bath. The pudding was cooked for 2 minutes while being stirred with 60 strokes per minute; it was covered and cooked for 15 minutes longer, being stirred with 10 strokes at the end of the 5-, 10-, and 15-minute periods. Immediately after the pudding was removed from the water bath its temperature was recorded.[c] Further treatment was dependent upon the method used for measuring the consistency.

Procedure for Pudding Series I. Five levels of cornstarch were used each of which was approximately 10% less than the one preceding. Calculated on the basis of the weight of cornstarch to milk, these were 4.56, 4.10, 3.69, 3.33, and 2.98 g. of cornstarch per 100 g. of milk. Two of these levels were above and 2 below the level of cornstarch in the basic formula. The proportions of other ingredients to the milk were unchanged.

The procedure after the puddings were cooked was as follows: Portions were poured into 2 custard cups (Pyrex No. 462), covered with filter papers over which were placed the covers of petri dishes as described by Knowles and Harris *(12)*, cooled at room temperature for one-half hour, and then stored in the refrigerator overnight. The following morning the relative firmness of the puddings was measured by the penetrometer with the cone released for 5 seconds.

Procedure for Pudding Series II. After a Brookfield Synchro-Lectric Viscometer became available, the study was expanded to include 9 levels of cornstarch, namely, 5.63, 5.06, 4.56, 4.10, 3.69, 3.33, 2.98, 2.69, and 2.41 g. per 100 g. of milk.

Since it was not feasible to make and test 18 puddings (9 from each of 2 kinds of milk) in one period, an experimental design *(6)* was chosen in which 4 puddings from each kind of milk were made in one day. A total of 8 replications of each level of cornstarch with each kind of milk was made in 18 cooking periods. The order in which the groups of 4 levels of cornstarch were used was randomized among the days and within the group of 4 used on any given day. This plan was used to equalize as far as possible effects from possible slight variations in milk or differences in temperature and humidity of the laboratory which might occur from day to day.

Later a ninth replication of each pudding was made upon which both viscometer and penetrometer readings were obtained.

The treatment after the puddings were cooked was as follows: The upper part of the double boiler was removed from the water bath and placed in the lower part of a

[c] Average temperature of the various finished puddings, referred to in this study, just after removal from source of heat used in cooking was 91.5°C. ± 1.5°C.

double boiler containing a measured amount of boiling water. The heat was turned off, the temperature of the pudding was noted, then the double boiler was placed in position and adjusted to the proper height under the stationary viscometer. The reading of apparent viscosity was taken at once.

The Brookfield Viscometer (Model RVF) operates on the principle of the measurement of "drag produced upon a spindle rotating at a definite constant speed when immersed in the test material" *(3)*. Therefore, it was necessary to measure the apparent viscosity of the puddings while they were still hot since the consistency of cornstarch pastes changes rapidly upon cooling, and, at lower temperatures, gel formation occurs which would interfere with the satisfactory operation of an instrument such as this type viscometer. The sleeve attachment was used for all viscosity determinations since the material to be tested was not transferred to the size beaker for which the instrument was calibrated.

Preliminary studies were made to determine the size of spindle and speed of rotation best suited for the puddings from each starch concentration. Spindles Nos. 2, 3, 4, and 5, and a speed of 10 r.p.m. were found satisfactory for the range of viscosity represented by these puddings.

pH of milk. The pH values of the milks used each day were determined with the Beckman pH meter.

RESULTS AND DISCUSSION

The pH values of the milk varied but slightly from day to day. The average values were 6.61 and 6.63 for the nonhomogenized and the homogenized milks, respectively.

The average penetrometer readings of the puddings made from the 2 kinds of milk and 5 levels of cornstarch in Series I are given in Table 1 and shown graphically in Figure 1.

It is apparent from Figure 1 that for levels of cornstarch above that used in the basic formula (3.69 g. per 100 g. milk), the curves for the 2

TABLE 1

Summary of average penetrometer readings on cornstarch puddings made with nonhomogenized and homogenized milk. Series I and II

Level of cornstarch	Penetrometer readings	
	Nonhomogenized milk	Homogenized milk
g./100 g. milk	*0.1 mm.*	*0.1 mm.*
Series I (five replications)		
2.98	345	346
3.33	326	312
3.69	301	281
4.10	278	259
4.56	258	243
Series II (only one replication)		
2.41	371[d]	353[d]
2.69	352[d]	358[d]
2.98	343	348
3.33	326	304
3.69	307	277
4.10	282	258
4.56	265	248
5.06	242	229
5.63	224	218

[d] These readings are not highly accurate because the puddings were too soft to get satisfactory readings with the penetrometer.

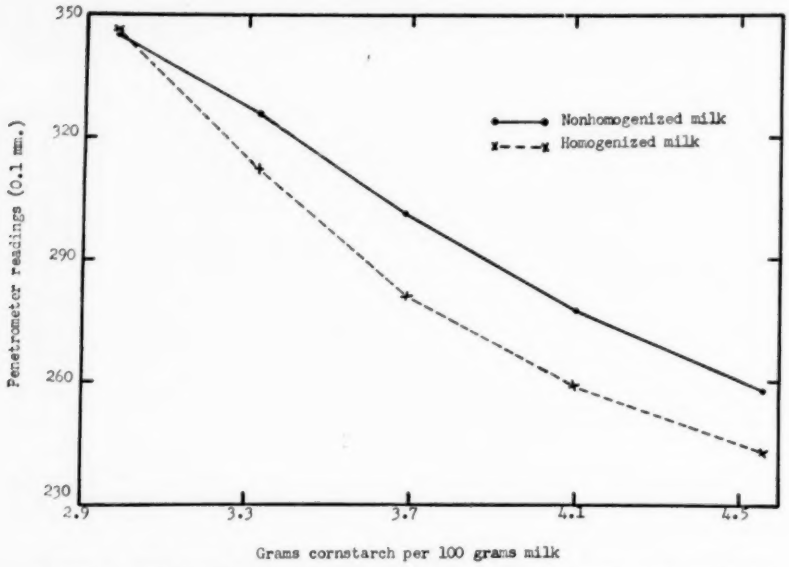

Figure 1. The average penetrometer readings of puddings made with nonhomogenized and homogenized milk and five levels of cornstarch.

kinds of milk are almost parallel, but for values less than the middle value, the curves tend to converge and at the lowest value of cornstarch used, the 2 lines intersect. It is, therefore, evident that any statement as to the percentage reduction in cornstarch needed to produce a consistency in homogenized milk puddings comparable to that in those made with nonhomogenized milk must be dependent upon the level of cornstarch in the pudding used as the standard for comparison.

From the graph, it may be estimated that the amount of cornstarch to use with homogenized milk to make a pudding of the consistency of a nonhomogenized milk pudding made with 3.69 g. per 100 g. of milk is about 3.44 g. of cornstarch which is slightly less than a 7% reduction.

In Table 2 are presented the average apparent viscosities of the puddings in Series II made from the 2 kinds of milk and 9 levels of cornstarch. Figure 2 depicts the results graphically. Since cornstarch puddings are non-Newtonian fluids at their cooking temperatures, the viscosity of the puddings has been expressed as apparent viscosity units rather than as centipoises.

An analysis of variance made upon the results of 9 replications for each of the 9 levels of cornstarch showed that at the lowest level of cornstarch used there was no significant difference in the viscosities of the puddings made from the 2 kinds of milk. At the next level, there was a slight difference; for all higher levels, the puddings made from homogenized milk were much more viscous than those from nonhomogenized milk. The differences were highly significant when tested statistically.

From the curves in Figure 2, it appears that if no more than approximately 3% of cornstarch, based upon the weight of milk, is used, only slight differences in the consistency of hot puddings from the 2 kinds of milk may be expected, but, for levels higher than 3%, the difference in consistency may be expected to become increasingly greater with each higher level of cornstarch. These conclusions apply to the conditions of this study; no assumption is made as to whether the difference would continue to increase if still higher levels of cornstarch were used.

TABLE 2

Averages of the apparent viscosities of cornstarch puddings made with nine levels of cornstarch and nonhomogenized and homogenized milk. (Nine replications in each average)

Level of cornstarch	Milk	
	Nonhomogenized	Homogenized
g./100 g. milk	*viscosity units*	*viscosity units*
2.41	87	85
2.69	145	173
2.98	255	430
3.33	522	1,943
3.69	1,233	3,716
4.10	2,534	5,674
4.56	4,242	7,949
5.06	5,980	10,370
5.63	8,791	13,593

The average penetrometer readings for the one set of puddings made in Series II are shown in Table 1. It will be observed that with the 2 highest levels of cornstarch, the difference between the readings for the 2 kinds of milk tended to become somewhat less; this is in contrast to the greater difference in apparent viscosity noted in the hot puddings.

From the data obtained in these 2 series, it is evident that above a certain minimum level of cornstarch, homogenized milk produced puddings of greater viscosity when hot and of a firmer gel when cold than did nonhomogenized milk from the same source.

The only variable in any pair of puddings compared was the type of milk used—in the one case nonhomogenized milk, in the other, milk from the same source homogenized at 2,500 pounds' pressure.

The chief difference between homogenized and nonhomogenized milk is in the dispersion of the fat. According to Trout *(20)*, in the process of homogenization the larger fat globules are subdivided into many smaller ones, and thereby the fat surface area is greatly increased. Therefore, it would seem that the difference in the consistency of the puddings made with the 2 kinds of milk must be associated with the surface area of the fat globules in the milk.

Few investigations dealing with the effect of natural fats on the viscosity of starch pastes were found in the literature. Whistler and Hilbert *(21)* stated: "Many . . . properties of cereal starch pastes, such as their opacity, viscosity, gelling power, retrogradation rate, and iodine absorption capacity are affected by fats." Lehrman *(13)* reported that starches adsorb saturated fatty acids in preference to the unsaturated and con-

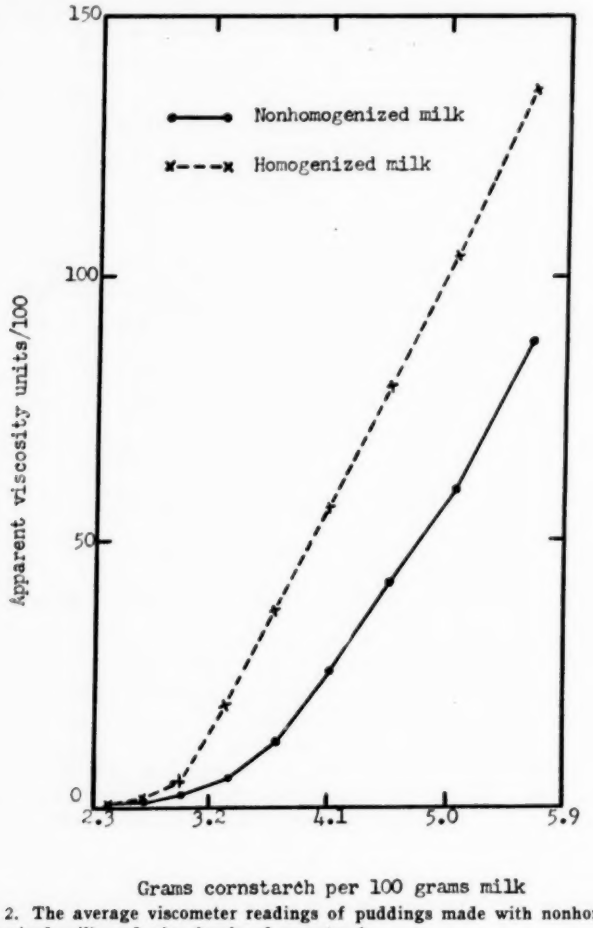

Figure 2. The average viscometer readings of puddings made with nonhomogenized and homogenized milk and nine levels of cornstarch.

cluded that fatty acids associated with starch are probably adsorbed. Bechtel *(1)* found that the defatting of cornstarch had the effect of lowering the maximum viscosity of unmodified cornstarch to about one-half of that of the original starch. Schoch *(18)* reported that some of the physical differences between tuber and cereal starches can be ascribed to the presence of polar fatty acids in the latter. He pointed out that the character of pastes made from cereal starches from which the fatty acids have been extracted differs markedly from that of pastes made from the natural starches.

Kerr *(10)* stated that the higher fatty acids, such as stearic, palmitic, and linoleic, all materially increase the hot paste viscosity of cornstarch when added to the starch in small amounts.

Heald *(8)*, in reporting the colloidal behavior of soap-starch systems, stated that the addition of certain measured amounts of sodium oleate to the starch paste produced an increase in the viscosity of the paste. This increase was thought to be due to the adsorption of the negative oleate ion by the starch. Kesler and Black *(11)* likewise observed that the addition of soap increased the viscosity of starch pastes.

Mitchell and Zillmann *(14)* found that fatty acids and their salts increased the viscosity of starch pastes made from non-waxy starches. They reported that among the factors which influenced the physical properties of the starch-fatty acid mixtures were the starch-water ratio and the amount of fatty material present as well as the length of the fatty acid chain.

From the reports of these investigators it appears, therefore, that fatty materials (soaps, fatty acids, and perhaps natural fats) are adsorbed by starch particles and, in certain proportions, produce an increased viscosity in starch pastes.

In the light of these reports and in consideration of the greater viscosity and gel firmness of cornstarch puddings made with homogenized milk as compared with similar ones from nonhomogenized milk, it was postulated that this difference in viscosity may be related to the relative surface area of the fat globules in the two kinds of milk. The increased surface area of the fat globules in homogenized milk increases the amount of fatty material exposed and available for colloidal reaction and may be considered equivalent to increasing the amount of fat in the starch paste.

PART II

In order to test the validity of the assumption expressed above, three experiments were conducted. Puddings were made (a) from skim milk to which varying amounts of fat were added, (b) from milk from two breeds of cows known to produce milk of different size fat globules, and (c) from low-fat homogenized milk calculated to have about the same fat surface area as nonhomogenized whole milk.

EXPERIMENTAL PROCEDURE

Puddings from skim milk. Early in the study a series of puddings was made from skim milk to which varying amounts of fat were added. These amounts were (a) an amount calculated to be the equivalent of that in normal whole milk of 4% fat content, (b) an amount equivalent to one-half that in whole milk, (c) an amount equal to one-fourth that of whole milk, and (d) no added fat. Clarified butter fat and refined cottonseed oil were used. The liquid fat was stirred into the pudding mixture at the beginning of the cooking period. A pudding from nonhomogenized whole milk was also made for comparison. The level of cornstarch of the basic formula (3.69 g. per 100 g. milk) was used. The relative consistency of the cold puddings was measured with the penetrometer.

Puddings from milk from Jersey and Holstein cows. It is known that fat globules from the milk of Jersey cows are larger in size than those from the milk of Holsteins *(16)*. Therefore, if the percentage of fat in the milk from the 2 breeds were the same, the milk having the smaller fat globules would have the greater surface area of fat; hence, the Holstein milk would resemble, to a degree, homogenized milk, and the Jersey milk, the nonhomogenized milk.

Milk from these two breeds was obtained. The fat content of the Jersey milk was adjusted to that of the Holstein; namely, 4.2%.

These 2 milks with the same percentage of fat but with different average size fat globules were used in puddings made with 3.69 g. cornstarch per 100 g. of milk. The viscosity of the hot puddings was measured with the viscometer, the firmness of the cold puddings with the penetrometer.

Puddings from low-fat homogenized milk. Since, according to Trout *(20)*, the process of homogenization increases the surface area of fat globules by 5 or 6 times, it was planned to have a sample of homogenized milk prepared with approximately one-sixth to one-fifth the fat content of normal whole milk.

Three lots of milk were obtained: nonhomogenized with fat content 4.3%; homogenized whole milk, fat content 4.1%; and homogenized milk with 0.6% fat. For making the puddings with these milks, the 3.69-g. level of cornstarch per 100 g. of milk was used. The apparent viscosities of the hot puddings and penetrometer readings on the cold puddings were taken.

RESULTS AND DISCUSSION

Results of all 3 of the experiments as shown in Table 3 lend support to the belief that the higher viscosity and greater firmness of homogenized milk puddings are associated with the greater surface area of fat globules. In the experiment with skim milk, the pudding from the nonhomogenized whole milk was firmest (288 penetrometer units); the one with butter fat added to skim milk in the proportion of that in whole milk was almost as firm (penetrometer reading, 290). The puddings made with smaller amounts of fat were less firm. Too few samples were prepared to show conclusively that added butterfat at each level produced greater firmness in the pudding than did an equal amount of oil, but the data indicated the probability of this being true.

TABLE 3

Penetrometer readings and apparent viscosity units for cornstarch puddings (3.69 g. cornstarch per 100 g. milk) made with milks treated to show the effect of different fat surface areas upon viscosity of the puddings

Kind of milk and treatment	Penetrometer reading	Apparent viscosity by Brookfield viscometer
	0.1 mm.	*viscosity units*
Skim milk		
No added fat	307	.....
Added fat, equivalent to whole milk		
Butter fat	290	.....
Oil	297	.....
Added fat equivalent to one-half that in whole milk		
Butter fat	300	.....
Oil	298	.....
Added fat equivalent to one-fourth that in whole milk		
Butter fat	297	.....
Oil	306	.....
Nonhomogenized whole milk	288	.....
Milk from two breeds of cows		
Jersey	299	1,260
Holstein	278	3,203
Nonhomogenized whole milk	307	1,065
Low-fat (0.6%) homogenized milk	309	1,075
Homogenized whole milk	287	4,240

It was observed that the fat in the skim milk puddings was not in an emulsified form at the end of the cooking period, but rather appeared as small globules visible throughout the pudding.

In the experiment in which Jersey and Holstein milks were used, the difference in the penetrometer readings of 278 for the puddings made with Holstein milk and 299 for those from Jersey milk was of the order found between those observed for homogenized and nonhomogenized milk puddings of Series I for the same level of cornstarch as presented in Table 1. Likewise, the viscometer readings of 1,260 and 3,203 viscosity units, respectively, for the hot puddings made from the Jersey and Holstein milks, were similar to the 1,233 and 3,716 viscosity units which were the average values for the 9 replications of puddings made with nonhomogenized and homogenized milk and the 3.69-g. level of cornstarch.

In the third experiment the 1,075 viscosity units for the hot pudding and the penetrometer reading of 309 for the cold pudding made from the low-fat homogenized milk agree very closely with the 1,065 viscosity units and the 307 penetrometer reading of the pudding made with the nonhomogenized whole milk. Since it was estimated that the surface area of the fat globules in the 0.6% fat content homogenized milk was about equal to the surface area of the globules in nonhomogenized whole milk, the results give strong support to the assumption that the greater viscosity and increased firmness of cornstarch puddings made with homogenized milk are related to the surface area of the fat in the milk.

As has been pointed out in the discussion of the results of Series I and II, the marked effect of the use of homogenized milk upon the viscosity and the firmness of the gel of cornstarch puddings was observed in those puddings in which the proportion of cornstarch to milk was 3 or more g. per 100 g. of milk. With the lowest levels of cornstarch used in this study there was little or no difference in the apparent viscosity of the hot puddings or the firmness of the cold puddings made from the 2 kinds of milk. Consequently, it must be assumed that below certain levels of cornstarch, an increased fat surface area ceases to be a factor in determining the viscosity of a starch pudding.

SUMMARY

Cornstarch puddings were prepared with nonhomogenized milk and milk from the same source homogenized at 2,500 pounds' pressure. Several different levels of cornstarch were used. The apparent viscosity of the hot puddings and the relative firmness of the cold puddings were measured by the use of a viscometer and penetrometer, respectively.

Puddings made with homogenized milk and the proportion of cornstarch to milk typical of household recipes were more viscous when hot and firmer when cold than similar puddings made from nonhomogenized milk.

The degree of difference in the viscosity and firmness of the puddings made with the 2 kinds of milk was found to vary with the concentration of the cornstarch. Under the conditions of this study, in those instances in which the proportion of cornstarch was 3 g. or more to 100 g. of milk, puddings made with homogenized milk were more viscous and of firmer consistency than those made from nonhomogenized milk from the same

source. However, for levels of cornstarch below 3 g. per 100 g. of milk, there was little difference in the viscosity of puddings made from the 2 kinds of milk. Consequently, there was no one value for the percentage reduction needed to produce a homogenized milk pudding of consistency similar to one made with nonhomogenized milk from the same source. The amount of reduction was dependent upon the level of cornstarch in the pudding being used as a standard of comparison.

Evidence was presented (through 3 separate experiments) to support the assumption that the difference in consistency in cornstarch puddings made with homogenized and nonhomogenized milks and the levels of cornstarch commonly used in this type of product is associated with and probably due to the difference in the surface area of the fat globules in the 2 kinds of milk.

LITERATURE CITED

1. Bechtel, W. G. A study of some paste characteristics of starches with the corn industries viscometer. *Cereal Chem.*, **24**, 200 (1941).
2. Brimhall, B., and Hixon, R. M. Interpretation of viscosity measurements on starch pastes. *Cereal Chem.*, **19**, 425 (1942).
3. Brookfield Engineering Laboratories, Inc. Instructions for care and operation of the Brookfield Synchro-Lectric Viscometer.
4. Caesar, G. V. Consistency changes in starch pastes. *Ind. Eng. Chem.*, **24**, 1432 (1932).
5. Carr, R. E., and Trout, G. M. Some cooking qualities of homogenized milk. I. Baked and soft custard. *Food Research*, **7**, 360 (1942).
6. Cochran, W. G., and Cox, G. M. *Experimental Designs*. 1950, John Wiley and Sons, Inc., New York, N. Y.
7. Gallay, W., and Bell, A. C. The viscosity of cornstarch pastes. *Can. J. Research*, **14B**, 360 (1936).
8. Heald, A. M. Colloidal behavior of soap-starch systems. *Paper Trade J.*, **113** (2), 39 (1941).
9. Hollender, H. A., and Weckel, K. G. Stability of homogenized milk in cookery practice. *Food Research*, **6**, 335 (1941).
10. Kerr, R. W. (Editor) *Chemistry and Industry of Starch*. 1944, Academic Press, Inc., New York, N. Y.
11. Kesler, C. C., and Black, W. C. A precipitated starch product. *Paper Trade J.*, **114** (21), 57 (1942).
12. Knowles, D., and Harris, R. H. Observations on the behavior of starch gels from different classes and varieties of wheat. *Food Research*, **8**, 409 (1943).
13. Lehrman, L. The nature of the fatty acids associated with starch. The adsorption of palmitic acid by potato and defatted corn and rice starches. *J. Am. Chem. Soc.*, **64**, 2144 (1942).
14. Mitchell, W. A., and Zillmann, E. The effect of fatty acids on starch and flour viscosity. *Trans. Am. Assoc. Cereal Chemists*, **9** (1), 64 (1951).
15. Morgan, W. L., and Vaughn, N. L. Starch viscosity or strength. *Ind. Eng. Chem.*, **38**, 233 (1943).
16. Rogers, L. A. (Associates of). *Fundamentals of Dairy Science*. 2nd ed., 1935, Reinhold Publishing Corporation, New York, N. Y.
17. Shaw, Elizabeth N. Studies on the use of homogenized milk in cookery. (Unpublished M.S.H.E. Thesis, 1952, Purdue University Library, Lafayette, Indiana.)
18. Schoch, T. J. Physical aspects of starch behavior. *Cereal Chem.*, **18**, 121 (1941).
19. Towson, A. W., and Trout, G. M. Some cooking qualities of homogenized milk. II. White sauces. *Food Research*, **11**, 261 (1946).
20. Trout, G. M. *Homogenized Milk*. 1950, Michigan State College Press, East Lansing, Mich.
21. Whistler, R. L., and Hilbert, G. E. Extraction of fatty substances from starch. *J. Am. Chem. Soc.*, **66**, 1721 (1944).

RELATIVE BACTERICIDAL EFFICIENCIES OF THREE TYPES OF HIGH-ENERGY IONIZING RADIATIONS*

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In sterilization by ionizing radiations many factors must be considered. One of the most important is the relative bactericidal efficiencies of the various types of radiation that may be available. As has been shown previously *(5, 9)*, only beta and gamma radiations, either from particle accelerators or from radioactive isotopes, deserve serious consideration as agents for sterilization. Although the literature is replete with articles on the individual effects of specific types of radiation on various organisms, it is notably lacking in articles on the relative bactericidal efficiencies of beta and gamma radiations under strictly comparable conditions of irradiation, dosimetry, and bacteriological techniques. Attempts to correlate the scattered information in the literature have revealed inconsistencies in the available published data, as well as the problems one might expect to encounter in carrying on such a comparative study.

In view of the recognized importance of obtaining comparable data before any evaluation of possible commercial application of "cold sterilization" can be made, it was felt imperative that an investigation be initiated to evaluate accurately the relative bactericidal efficiencies of beta and gamma radiations. Having access to a Van de Graaff accelerator producing both cathode (beta rays) and gamma (X-rays) radiations, as well as a multi-curie isotopic gamma emitter (Cobalt-60), the Department of Food Technology at the Massachusetts Institute of Technology has unique facilities for the conduct of such a comparative study. The present investigation, therefore, was undertaken to compare the relative bactericidal efficiencies of these 3 types of radiation in experiments on 2 species of bacteria, one a non-spore-forming organism *(Escherichia coli)* and the other a spore-forming organism, *Bacillus thermoacidurans (B. coagulans)*.

Before an attempt is made to discuss the relative bactericidal efficiencies of radiations that differ in such characteristics as particle size, specific ionization along the path, and uniformity of ionization distribution, it would be well to define the standard of comparison. From all points of view, it would appear that this standard should be *equal quantities of energy absorbed per gram of sample* irradiated by the different types of radiation under study. In essence this statement infers that each sample will receive the same dose in rep units. However easy it may be to make such a statement, it is much more difficult to specify valid conditions to insure this experimentally. This difficulty stems from the dosimetric measurements employed.

*Contribution No. 164 from the Department of Food Technology, Massachusetts Institute of Technology, Cambridge, Mass.

In any research of this type, the following points pertaining to dosimetry should be carefully considered:

(a) In the calculation of dose, if the sensitive portion of the dosimeter differs in composition from that of the actual sample (e.g., if an air-ionization chamber is used for dosimetry and aqueous samples of material are under study), careful application of theoretical and experimental knowledge must be made in the transition from the calculations for ionization in air to the calculations for that in liquid.

(b) The dosimeters should approximate as closely as possible the actual samples in material and geometry, to duplicate absorption and scattering characteristics.

(c) Dosimeters of both the physical and the chemical type yield dose values that are the arithmetical average of doses received over the entire sensitive volume of the dosimeter. For example, the dose determined by the dosimeter may be represented by Eq. 1:

$$D_{dosimeter} = \frac{1}{V} \sum D_i \Delta v_i \qquad (1)$$

where V is the sensitive volume of the dosimeter and D_i is the dose received by a volume element $\triangle v_i$.

If the ionization density is uniform (e.g., in a cylindrical Cobalt-60 source), $D_{dosimeter}$ is equal to D_i, that is, the same dose is received at all points in the sample. If the ionization density is not uniform (e.g., cathode rays), then a critical examination of the arithmetical mean dose under the particular conditions of study is necessary to ascertain whether the arithmetical mean dose can be validly used to describe the results obtained. This will be discussed in detail later.

EXPERIMENTAL PREMISES

Radiation equipment. A Van de Graaff accelerator,[b] producing monoenergetic beams of electrons up to and including 3 m.e.v., was used as the source of X-rays and cathode rays *(11)*. In the cathode-ray experiments, samples were exposed to the electron beam on a moving belt directly under the beam. In the X-ray experiments, samples to be exposed were held rigidly in place by a special Lucite sample holder (bolted to the generator) capable of holding a maximum of 15 samples (Figure 1). The gamma radiations came from a kilocurie Cobalt-60 source of hollow cylindrical shape *(7, 8)*. Figure 2 represents a cross-section of the shield and source with a sample carrier in place. The sample carrier is constructed of Lucite and allows for the simultaneous irradiation of 4 ampoules.

Both the Van de Graaff accelerator and the Cobalt-60 source were carefully and precisely calibrated by chemical and physical means.

Irradiation vessels. In all the experiments with gamma radiation from the Cobalt-60 source and in the majority of the experiments with the Van de Graaff accelerator, 5-ml. Kimble, thin-walled glass ("Neutraglas") ampoules were used as irradiation vessels. In the Cobalt-60 source, the ampoules were irradiated in the vertical position, whereas with the Van de Graaff accelerator they were irradiated in the horizontal position.

In the cathode-ray studies, for reasons discussed in detail later, a number of experiments were performed in which Petri dishes were used as irradiation vessels, in order

[b] The writers are grateful for the continuing cooperation of Prof. John G. Trump and Mr. K. A. Wright of the Electrical Engineering Department, Massachusetts Institute of Technology, in making the Van de Graaff accelerator available for use in these experiments.

to obtain samples of uniform thickness. These dishes were 8.9 cm. and 5.0 cm. in diameter.

Dosimetry. The Cobalt-60 source was calibrated for the 4 different positions in the sample carrier in which samples were irradiated, both chemical and physical methods of dosimetry being used. These dosimetric methods have recently been discussed in detail *(2)*. The calorimeter used for the calibration by physical dosimetry was designed to have geometrical and absorber characteristics similar to those of an ampoule sample in the Lucite carrier. The calibrations by chemical dosimetry were made with ampoules identical with those used for bacterial samples.

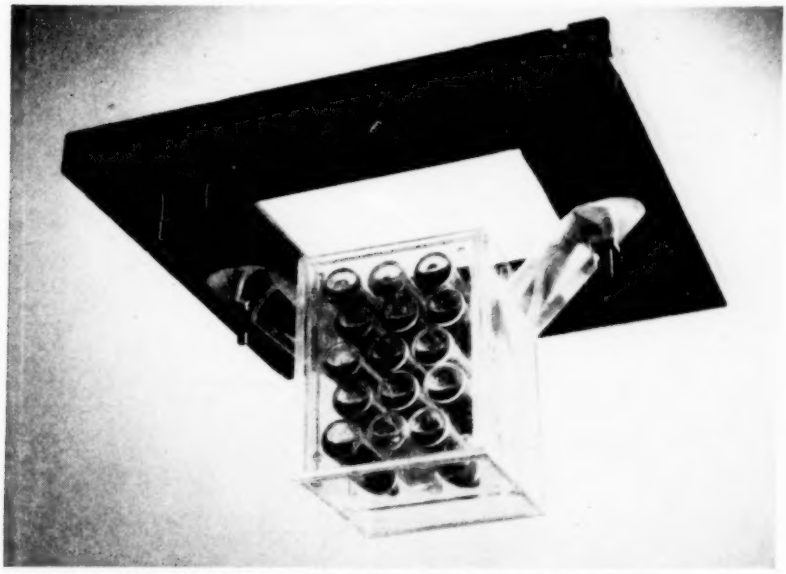

Figure 1. Lucite sample holder used in X-ray experiments.

The Van de Graaff accelerator had previously been calibrated by power measurements. In addition, for the cathode-ray studies described in this report ampoules containing ferrous-ferric *(2, 10)* dosimeter solutions of identical volumes were taped to the ampoules containing the bacterial cultures, so that samples and dosimeters lay side by side on the belt. The dose delivered to the sample was therefore considered to be the average dose as read by the companion dosimeter, for the electron beam was distributed uniformly in the lateral direction. The Petri dishes, because of their comparatively large diameter, were arranged in a single line and contained alternately samples and dosimeters (volumes identical with those of the samples). The dose delivered to a sample under these conditions was considered to be the average of the doses received by the dosimeters on the belt in front of and behind the sample.

The doses of X-rays delivered by the accelerator to the various positions of the sample holders were determined from previous calibrations where dosimeter ampoules were placed in all the positions and the relative intensity of dose was calculated for each position. In the runs with bacterial samples, 2 dosimeter ampoules were used in each run to determine the dose for 2 positions; the doses for the other positions were calculated from the previous measurements of relative intensity of dose.

Thus for each exposure of bacteria to radiations produced by the particle accelerator, careful dosimetric checks were made in irradiation vessels identical with those in which the test organisms were exposed. In the case of experiments with the gamma-emitting

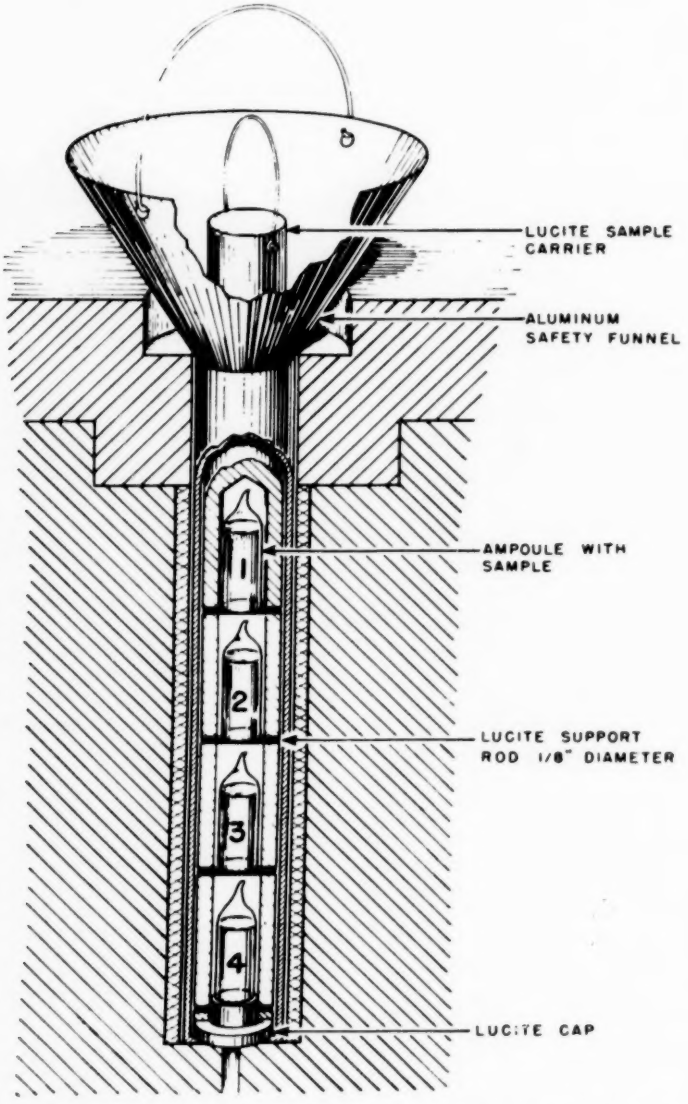

Figure 2. Cross section of shield and Cobalt-60 source with sample carrier in place.

isotope, which was carefully calibrated by physical and chemical techniques for each irradiation position (with correction for decay), the only variable to be controlled was that of length of exposure.

Bacteriological techniques. Standard bacteriological techniques were used throughout. With *E. coli*, 24-hour cultures were grown in nutrient broth at 37°C. Aliquots of this 24-hour nutrient broth suspension were irradiated. After irradiation and after a 48-hour incubation period at 37°C., standard plate counts were made.

Spores of *B. thermoacidurans* were obtained by inoculating Thermoacidurans Agar (Difco) in Roux flasks and incubating them at 55°C. for 10 to 12 days. The cells were then suspended in physiological saline solution, harvested, and heated for 10 minutes at 100°C. to destroy the vegetative cells. This stock of spores was kept in the refrigerator, and aliquots were taken as needed. After irradiation, duplicate 1-ml. aliquots were aseptically transferred by pipette from each irradiated ampoule (or dish) to dilution blanks, diluted, plated on Thermoacidurans Agar (Difco), and incubated at 55°C. for 48 hours.

Theoretical discussion of effect of uniformity of ionization density. It is essential to examine the relative differences between beta and gamma radiations insofar as the absorber is concerned, before proceeding to any quantitative assessments of the effects of these radiations. The outstanding and obvious difference (other than depth of penetration) between gamma rays emitted from a Cobalt-60 source and X-rays and cathode rays produced by a particle accelerator is the ionization pattern created in the absorber. Experimental evidence and theoretical speculation regarding ionization density in the particular type of tubular Cobalt-60 source used in this investigation indicate a uniform distribution of dose over the entire volume of the sample *(12)*. However, in contrast to γ-rays, the intensity distribution of cathode rays in matter is non-uniform. Moreover, cathode rays have a finite range in matter, which is dependent upon the energy levels *(3)*, whereas γ-rays show an exponential absorption and have relatively greater penetration.

Figure 3 presents the relative ionization densities of 1, 2, and 3 m.e.v. electrons in matter. With 3 m.e.v. cathode rays, for example, the curve for relative ionization rises from a level of 60% to a maximum of 100% at an absorber thickness of 0.55 g. per sq. cm. and then rapidly falls off to zero. In other words, even for a sample having a thickness of only 0.55 g. per sq. cm. there will be a variation of as much as 40% in

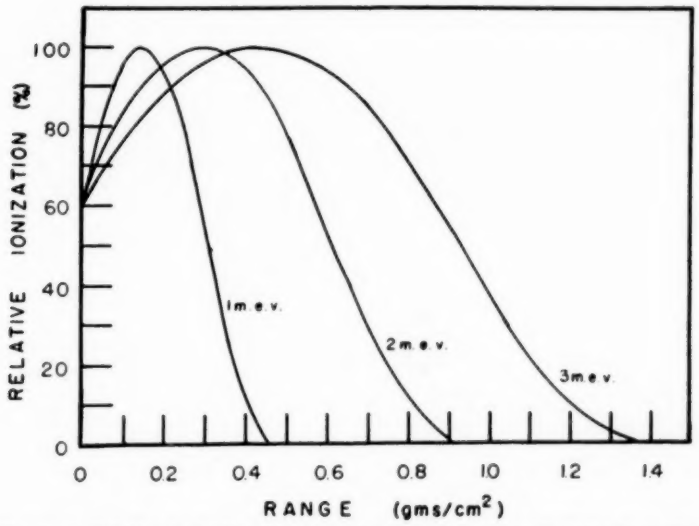

Figure 3. Relative ionization densities of 1, 2, and 3 m.e.v. electrons in matter.

the ionization density within the sample. Therefore, the question might well be raised whether the variation in ionization density of cathode rays may not be of significance in bacteriological studies comparing cathode rays with gamma rays, since the latter type of radiation results in a uniform or homogeneous radiation distribution within the absorber and one is interested in the relative bactericidal effects resulting from the absorption of equal average amounts of energy of each type of radiation, as defined previously.

The simple target theory formulation *(6)* offers no problem in its application to a beam of radiation of uniform ionization, because the dose D is constant throughout (Eq. 2):

$$\frac{n}{n_o} = e^{-D/D_o} \tag{2}$$

However, in the case of a non-uniform radiation beam, such as high-energy cathode rays, D is not constant throughout the volume of the absorber. Therefore, the above equation can be applied only to elements of small volume, Δv_i, small enough so that the dose D_i is essentially constant over the element.[c] Under these conditions, Eq. 2 can be applied to elements of k volume and the separate equations for k can be summated to give Eq. 3:

$$n = \sum_{i=1}^{k} n_i = \frac{n_o}{V} \sum_{i=1}^{k} e^{-D_i/D_o} \Delta v_i \tag{3}$$

Eq. 3 may be made more useful by replacing the summation form with an integration form and replacing the volume elements with linear distance elements dx, where x is now the distance penetrated into the sample (g. per sq. cm.). The latter assumption is valid so long as there is no variation of ionization density laterally. Eq. 3 then becomes:

$$n = \frac{n_o}{x_o} \int_{x=o}^{x=x_o} e^{-D/D_0} dx \tag{4}$$

where $\frac{n}{n_o}$ is the fraction of organisms surviving a dose D, D_o is the 37% dose, x is the linear penetration, and x_o the thickness at the maximum dose (D_{max}) depth at which maximum ionization occurs.

Eq. 4 represents a summation of survivors over sheets of infinitesimal thickness dx perpendicular to the beam. If D is constant for different values of x (homogeneous radiation beams), then Eq. 4 reduces to Eq. 2, as illustrated in Figure 4. This assumes that the concentration of bacteria is initially uniform and that there is no diffusion of bacteria during an irradiation run. (In the case of cathode rays, maximum exposure times of 1.5 seconds have been used, and therefore this assumption appears valid.)

If the dependence of D on x is assumed to be linear, Eq. 4 can be easily integrated. If the entrance dose is defined as D_e (where $x = 0$), then the dose at any given point x is shown by Eq. 5:

$$D = D_e + bx \tag{5}$$

where it is assumed that the curve between $x = 0$ and $x = x_o$ is a straight line of slope b. The maximum dose at $x = x_o$ may be represented in Eq. 6:

$$D_{max} = D_e + bx_o \tag{6}$$

By substitution of Eq. 5 in Eq. 4 and integration of Eq. 4, one obtains Eq. 7:

$$n = \frac{n_o D_o}{bx_o} \left(e^{-D_e/D_o} - e^{(-D_e + bx_o)/D_o}\right) \tag{7}$$

[c] The subscript i refers to the i-th element taken.

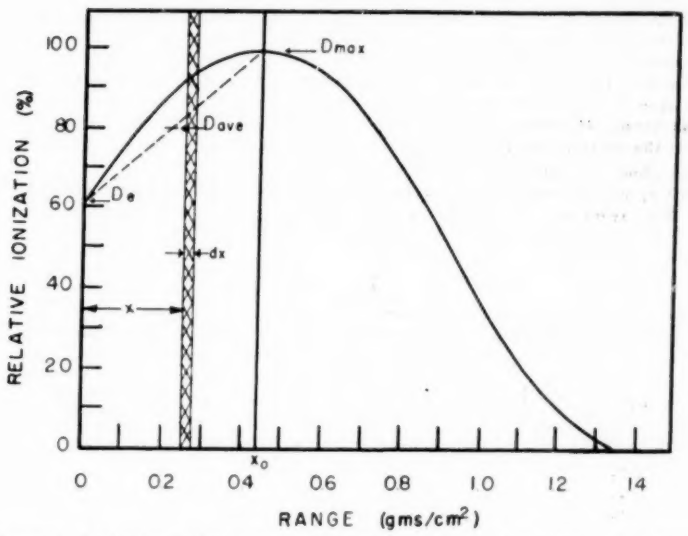

Figure 4. Ionization-in-depth curve for non-homogeneous ionization. (One straight-line approximation used for calculation of dose distribution.)

By substitution of Eq. 6 in Eq. 7, one obtains:

$$n = n_o \frac{D_o}{D_{max} - D_e} \left(e^{-D_e/D_o} - e^{-D_{max}/D_o}\right) \tag{8}$$

In order to calculate the order of magnitude of the discrepancy between the uniform and the non-uniform cases, numerical values may be approximated so that the average dose (D_{ave}), which is the dosimetrically determined dose, may be used. From Figure 4,

$$D_{max} = (5/3)D_e \tag{9}$$

$$D_{ave} = (4/3)D_e \tag{10}$$

Therefore, for the uniform case, Eq. 2 becomes:

$$n = n_o e^{-(4/3)D_e/D_o} \tag{11}$$

and for the non-uniform case, Eq. 8 becomes:

$$n = n_o \frac{D_o}{(2/3)D_e} \left(e^{-D_e/D_o} - e^{-(5/3)D_e/D_o}\right) \tag{12}$$

By assigning different values for the ratio of the entrance dose to the mean lethal dose (D_e/D_o), Eqs. 11 and 12 may be compared. Eqs. 11 and 12 have been solved for different ratios of D_e/D_o, and some of these data are presented in Table 1.

These calculations indicate that the discrepancy between non-uniform and uniform radiations should not be great until the dose administered is approximately 4 times the

mean lethal dose for the given bacterial species. As the discrepancy becomes increasingly greater at higher dose levels and as sterility doses of the order of 2×10^6 rep are approximately 40 times the 37% dose for many spore-formers (theoretically, at least), it is apparent that in the so-called sterility region, for equal average doses of radiation (such as measured by dosimeters), there should be a much greater number of survivors in the case of the non-uniform radiation beams than in that of the uniform or homogeneous beams. Moreover, the apparent 37% dose for the 2 cases will differ by about 20%, on the basis of the same average dose.

One should be able to confirm the above 2 points experimentally by exposing to high-energy cathode rays samples varying from very thin to relatively great thickness. With thin layers of culture, one would expect to obtain results with cathode rays

TABLE 1

Theoretical comparison of survivors (n) and apparent D_o values for samples given the same average dose with uniform and non-uniform distributions of ionization density

D_e/D_o	$\frac{n(\text{non-uniform})}{n(\text{uniform})}$	$\frac{D_o(\text{non-uniform})}{D_o(\text{uniform})}$
Based on Eqs. 11 and 12		
0.25	1.00	1.00
0.5	1.00	1.00
0.75	1.01	1.01
1	1.02	1.01
1.5	1.04	1.02
2	1.07	1.03
3	1.17	1.04
4	1.35	1.06
6	1.81	1.08
8	2.69	1.09
10	4.21	1.12
20	58.9	1.18
40	2280	1.23
Based on Eqs. 13 and 14		
1	1.03	1.02
2	1.12	1.04
3	1.30	1.06
4	1.57	1.08
6	2.60	1.12
10	9.89	1.18
20	500	1.27
40	2.33×10^6	1.34

equivalent to those that might be obtained with homogeneous radiations. Therefore, at a given high ratio of D_e/D_o, lower D_o values and fewer survivors should be obtained for thin samples than for thick samples.

These theoretical considerations have been experimentally verified, and the details are reported in the subsequent experimental portion of this paper.

By fitting a smooth analytical curve to the experimental curve for ionization distribution of cathode rays (Figure 5), it is possible to obtain a more accurate expression for dependence of survival fraction upon dose than the expression (Eq. 12) derived above (Figure 4) where a straight line was used as a first-order approximation. Integration of the target theory expression (Eq. 2) by use of a smooth curve was attempted but proved to be too complex for numerical calculation of results in a reasonable time. A good approximation to the curve was obtained by fitting 3 separate straight-line

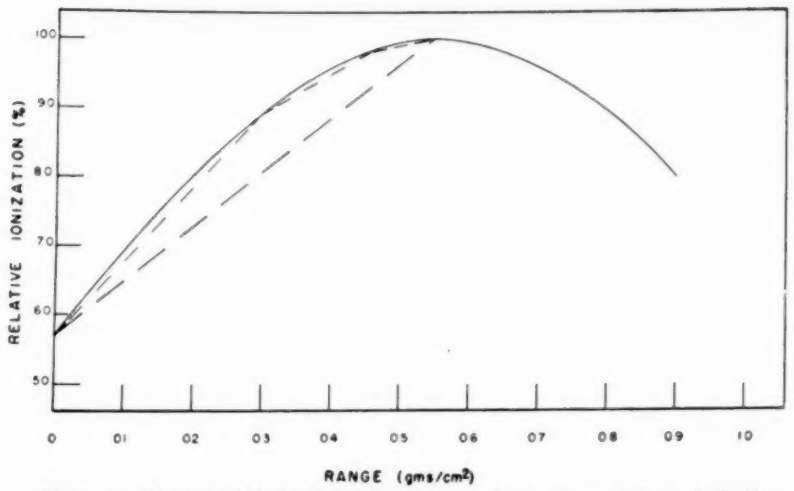

Figure 5. Portion of ionization-in-depth curve for non-homogeneous ionization. (Three straight lines used for approximation.)

segments to the experimental curve, as shown in Figure 5. The terminal points for the lines were taken at 57, 89, 98, and 100% relative ionization for ranges 0, 0.30, 0.45, and 0.55 g. per sq. cm., respectively. The resulting expressions are:

for non-uniform ionization

$$n/n_o = \frac{1.036}{a}\left\{0.938\left[e^{-a} - e^{-1.56140a}\right] + 1.667\left[e^{-1.56140a} - e^{-1.71929a}\right] + 5.00\left[e^{-1.71929a} - e^{-1.75438a}\right]\right\} \quad (13)$$

for uniform ionization

$$n/n_o = e^{-1.46172a}\ ;\ (D_{ave} = 1.46172\ D_e) \quad (14)$$

Ratios of survivors and apparent D_o values for the uniform and non-uniform cases calculated from Eqs. 13 and 14 are presented in the lower half of Table 1. The divergence between the 2 distributions is seen to become even more pronounced in this instance, resulting in a ratio of about 2×10^6 for the numbers of survivors and a difference of about 30% in the apparent D_o values at $D_e/D_o = 40$.

EXPERIMENTAL

Effect of gamma rays from Cobalt-60 on E. coli. In 1952 our initial experiments on the effects of gamma rays emitted by Cobalt-60 *(4)* were made on 5-ml. aliquots of a 24-hour suspension of *E. coli* in nutrient broth in ampoules (Table 2). In 1953, a second series of experiments was made with 3-ml. aliquots in ampoules (Table 3). In these 2 series of experiments, made one year apart with only the volume of the aliquot as the

variable (other than chronological time), 2 different inactivation doses (D_o values) were obtained, viz.:

3 ml. 6.49×10^3 rep_{DA}

5 ml. 10.1×10^3 rep_{DA}

To confirm the tentative conclusion that the difference in the D_o values for the 2 sets of data was caused by the change in the aliquot volumes rather than by any possible change in experimental conditions resulting from a year's lapse in time, a third series of experiments was conducted in which 3-ml. and 5-ml. aliquots of the *same broth cultures* were exposed simultaneously, in ampoules. The experiments in this series were repeated over a period of several days. The data (Table 4) confirm the volume effect found in the earlier experiments, as is indicated by the weighted mean D_o values shown at the bottom of Tables 2 to 4.

TABLE 2

Effect of gamma radiation from Cobalt-60 on E. coli in nutrient broth (5 ml./ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{DA} \times 10^{-4}$)	Mean D_o ($rep_{DA} \times 10^{-3}$)
3/18/52	15	0.82-4.11	11.8
3/19/52	15	0.82-4.11	8.63
3/20/52	11	0.82-4.93	8.50
3/21/52	17	0.82-4.93	8.65
3/24/52	12	0.82-4.93	10.6
3/25/52	12	0.82-4.93	10.1
3/26/52	12	0.82-4.93	10.4
3/27/52	12	0.82-4.93	8.96
3/28/52	12	0.82-4.93	13.5
	118 total		

Weighted mean $D_o = 10.1 \times 10^3$ rep_{DA}.

TABLE 3

Effect of gamma radiation from Cobalt-60 on E. coli in nutrient broth (3 ml./ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{DA} \times 10^{-4}$)	Mean D_o ($rep_{DA} \times 10^{-3}$)
2/18/53	16	0.65-2.90	5.92
2/27/53	5	3.21-3.62	6.29
3/3/53	20	1.28-2.14	6.03
3/4/53	12	1.94-2.18	6.39
3/5/53	16	1.92-3.58	5.20
3/6/53	15	1.92-3.58	6.50
3/9/53	4	0.64-0.715	4.19
3/12/53	5	3.20-3.57	6.14
3/13/53	8	0.64-0.72	5.10
3/19/53	22	0.64-8.86	7.25
3/26/53	20	0.64-4.29	8.78
	143 total		

Weighted mean $D_o = 6.49 \times 10^3$ rep_{DA}.

Effect of 3 m.e.v. cathode rays on E. coli. Aliquot samples (each of 3 ml.) of a suspension of *E. coli* in nutrient broth were placed in ampoules and bombarded by 3 m.e.v. cathode rays. The data (Table 5) confirm results obtained in these laboratories a year previously with cathode rays.

Effect of 1.6 m.e.v. X-rays on E. coli. To test the accuracy of the assumption that X-rays and gamma rays of the same energy levels have the same bactericidal effects, samples of a suspension of *E. coli* were exposed to 1.6 m.e.v. X-rays in ampoules (3 ml. per ampoule) in the sample holder mentioned previously (Table 6).

TABLE 4

Effect of gamma radiation from Cobalt-60 on E. coli in nutrient broth (3 ml. and 5 ml./ampoule)

Date of experiment	Ml. in ampoule	No. of samples irradiated	Dose range ($rep_{bio} \times 10^{-4}$)	Mean D_o ($rep_{bio} \times 10^{-4}$)
4/10/53	3	8	1.89-2.12	8.73
4/10/53	5	8	1.89-2.12	11.6
4/10/53	3	8	3.15-3.52	9.79
4/10/53	5	8	3.15-3.52	11.4
4/15/53	3	12	3.15-3.52	4.59
4/15/53	5	15	3.15-3.52	7.79
4/16/53	3	13	3.15-3.52	5.01
4/16/53	5	15	3.15-3.52	7.44
4/21/53	3	4	1.89-2.12	6.00
4/21/53	5	4	1.89-2.12	12.3
4/24/53	3	4	1.89-2.12	6.88
4/24/53	5	8	1.89-2.12	13.8
4/24/53	3	4	3.15-3.52	6.13
4/24/53	5	8	3.15-3.52	12.0
		119 total		

Weighted mean D_o (3 ml. samples) = 6.35×10^3 rep_{bio}.
Weighted mean D_o (5 ml. samples) = 10.1×10^3 rep_{bio}.

TABLE 5

Effect of 3 m.e.v. cathode rays on E. coli in nutrient broth (3 ml./ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{bio} \times 10^{-4}$)	Mean D_o ($rep_{bio} \times 10^{-2}$)
2/16/53	11	3.67	9.53
2/20/53	15	3.57	8.95
2/25/53	8	3.39-3.78	6.06
2/27/53	8	4.78-6.12	6.19
3/4/53	12	4.20-4.83	4.55
4/8/53	7	2.88-3.39	7.45
	61 total		

Weighted mean $D_o = 7.48 \times 10^2$ rep_{bio}.

TABLE 6

Effect of 1.6 m.e.v. X-rays on E. coli in nutrient broth (3 ml./ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{bio} \times 10^{-4}$)	Mean D_o ($rep_{bio} \times 10^{-2}$)
1/26/53	10	1.43-3.59	7.16
1/30/53	10	1.62-3.16	8.17
2/2/53	13	1.72-3.42	4.29
2/4/53	20	1.56-3.27	3.42
2/6/53	19	1.02-2.00	4.82
2/9/53	12	1.39-2.66	7.52
2/11/53	24	1.20-2.32	6.64
	108 total		

Weighted mean $D_o = 5.64 \times 10^2$ rep_{bio}.

Effect of 3 m.e.v. cathode rays on suspensions of E. coli of varying thickness. In the theoretical discussion presented previously, it was indicated that an average value for the dose received by a sample exposed to high-energy cathode rays might best be used to determine the true D_o value if the sample were thin. Moreover, it was indicated that the D_o values obtained with thin samples should be essentially equal to those obtained under conditions of uniform ionization such as occurs when gamma rays are used. Therefore, one should be able to compare the relative bactericidal efficiencies of cathode rays and gamma rays when a very thin suspension of organisms is to be bombarded by cathode rays. To test this hypothesis experimentally, samples of thicknesses ranging from 0.08 cm. to 0.5 cm. were exposed to 3 m.e.v. cathode rays. To achieve these thicknesses, aliquots of nutrient broth cultures of *E. coli* were pipetted into small and large Petri dishes. Petri dishes were selected because they are flat-bottomed and, therefore, enable one to obtain samples of uniform thickness, which is not possible with ampoules. Into small Petri dishes 5 cm. in diameter 2-ml. and 10-ml. aliquots (Table 7) of nutrient broth cultures were pipetted, and into large Petri dishes 8.9 cm. in diameter 5-ml. aliquots (Table 8) were pipetted. These samples had the following uniform thicknesses:

Sample	Thickness
5 ml.	0.081 cm.
2 ml.	0.102 cm.
10 ml.	0.51 cm.

The data in Tables 7 and 8 demonstrate that the mean D_o value increases with increasing thickness of culture. Therefore, it appears that for cultures of *E. coli*

TABLE 7

Effect of 3 m.e.v. cathode rays on E. coli in nutrient broth (2 ml. and 10 ml./small Petri dish)

Date of experiment	Ml. in Petri dish	No. of samples irradiated	Dose range ($rep_{Se} \times 10^{-4}$)	Mean D_o ($rep_{Se} \times 10^{-3}$)
4/3/53	10	12	2.27-2.60	7.99
4/3/53	2	13	2.30	5.55
4/8/53	10	11	3.95-4.62	15.4
4/8/53	2	12	2.96-4.69	7.13
4/13/53	10	10	3.15-3.57	12.3
4/13/53	2	9	3.15-3.39	5.70
4/15/53	10	10	6.46-6.96	9.00
4/15/53	2	10	6.13-6.78	7.03
4/27/53	10	9	2.57-2.73	6.12
4/27/53	2	8	2.57-2.80	5.49
		104 total		

Weighted mean D_o (10 ml. samples) = 10.3×10^3 rep_{Se}.
Weighted mean D_o (2 ml. samples) = 6.21×10^3 rep_{Se}.

TABLE 8

Effect of 3 m.e.v. cathode rays on E. coli in nutrient broth (5 ml./large Petri dish)

Date of experiment	No. of samples irradiated	Dose range ($rep_{Se} \times 10^{-4}$)	Mean D_o ($rep_{Se} \times 10^{-3}$)
3/9/53	5	4.51	4.19
3/11/53	9	4.61-6.09	4.93
3/16/53	8	5.19	5.50
3/25/53	10	7.99	7.29
4/1/53	22	5.13-6.02	4.54
	54 total		

Weighted mean D_o = 5.23×10^3 rep_{Se}.

irradiated by 3 m.e.v. cathode rays the D_o value depends directly upon the thickness rather than upon the volume of the sample. This was the result predicted by the theoretical derivation presented earlier.

Effect of gamma radiation from Cobalt-60 on B. thermoacidurans. To study the possibility of a volume effect with an entirely different type of bacterial cell, such as a spore-forming organism, 3-ml. samples of spores of *B. thermoacidurans* suspended in physiological saline solution were exposed in ampoules to the gamma radiations from Cobalt-60 (Table 9). With the data thus obtained as a control, comparative experiments were made with 3-ml. and 5-ml. samples from the same suspension of spores. These samples were irradiated at the same time in the Cobalt-60 source (Table 10).

TABLE 9

Effect of gamma radiation from Cobalt-60 on B. thermoacidurans in saline solution (3 ml./ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{air} \times 10^{-4}$)	Mean D_o ($rep_{air} \times 10^{-3}$)
3/2/53	7	8.95-10.3	45.0
3/5/53	8	9.60-10.7	41.6
3/10/53	11	3.20-10.7	33.4
3/12/53	8	9.60-10.7	42.1
3/13/53	8	3.20-3.57	49.4
3/17/53	23	3.28-7.15	41.3
3/20/53	20	6.14-14.3	53.9
3/31/53	20	9.60-28.6	46.3
4/2/53	20	9.45-28.2	48.3
	125 total		

Weighted mean $D_o = 45.3 \times 10^3$ rep_{air}

TABLE 10

Effect of gamma radiation from Cobalt-60 on B. thermoacidurans in saline solution (3 ml. and 5 ml./ampoule)

Date of experiment	Ml. in ampoule	No. of samples irradiated	Dose range ($rep_{air} \times 10^{-4}$)	Mean D_o ($rep_{air} \times 10^{-3}$)
4/14/53	3	4	9.45-10.6	41.5
4/14/53	5	4	9.45-10.6	43.7
4/14/53	3	4	18.9-21.2	47.6
4/14/53	5	4	18.9-21.2	47.8
4/17/53	3	4	9.45-10.6	44.0
4/17/53	5	4	9.45-10.6	43.0
4/17/53	3	4	18.9-21.2	44.9
4/17/53	5	4	18.9-21.2	46.1
5/1/53	3	8	9.45-10.6	47.0
5/1/53	5	4	9.45-10.6	45.0
5/1/53	3	8	18.9-21.2	51.2
5/1/53	5	2	18.9-21.2	51.1
		54 total		

Weighted mean D_o (3 ml. samples) $= 46.7 \times 10^3$ rep_{air}.
Weighted mean D_o (5 ml. samples) $= 47.2 \times 10^3$ rep_{air}.

The mean D_o value for the 3-ml. samples in the second series of experiments agrees well with the mean D_o value for the 3-ml. samples in the control series. Moreover, there appears to have been no volume effect in the case of *B. thermoacidurans*, as the mean D_o value for the 5-ml. samples is practically identical with that for the 3-ml. samples.

Effect of 3 m.e.v. cathode rays on B. thermoacidurans. A study of the effect of high-energy cathode rays on *B. thermoacidurans* was also made, the organisms being exposed

to the rays in ampoules, each containing 3 ml. of the suspension. A mean D_0 value of 50.1×10^3 rep_{60} was obtained (Table 11).

Effect of magnitude of dose rate. In any comparative study of the effects of different types of ionizing radiations on matter, the magnitude of the dose rate might conceivably be a factor in the effects noted. It was, therefore, deemed desirable to compare the survival ratios of organisms exposed to ionizing radiations at greatly different dose rates. With an istopic source of radiations it is difficult to do this unless a huge quantity of the isotope is available to enable a large difference in dose rates. With a particle accelerator as the source of radiations, however, it is easy to vary the beam current and thereby obtain dose rates that may differ a thousandfold or more. Experiments were, therefore, made in which samples of *E. coli* were exposed to radiations from a Van de Graaff accelerator (a) at a slow dose rate equivalent to that obtained from a kilocurie Cobalt-60 source, namely, of the order of magnitude of 2,000 rep_{60} per minute and (b) at a thousandfold faster dose rate of the order of 3,000,000 rep_{60} per minute.

TABLE 11

Effect of 3 m.e.v. cathode rays on B. thermoacidurans in saline solution (3 mL/ampoule)

Date of experiment	No. of samples irradiated	Dose range ($rep_{60} \times 10^{-4}$)	Mean D_0 ($rep_{60} \times 10^{-3}$)
2/25/53	8	8.90	37.2
3/2/53	8	13.1-13.8	62.1
3/16/53	8	8.92	42.5
3/18/53	8	9.82-20.6	63.9
3/23/53	11	9.4 -38.4	59.6
3/30/53	22	14.1-36.6	46.6
4/6/53	23	8.35-39.9	48.7
4/17/53	30	7.22-14.9	49.2
	118 total		

Weighted mean $D_0 = 50.1 \times 10^3$ rep_{60}.

Analysis of the data obtained (Table 12) indicates no difference whatever in the bactericidal efficiencies of the slow and rapid dose rates. These dose rates were such that 30 minutes of exposure at the slow dose rate were required as compared with one second of exposure at the rapid dose rate in order to achieve total doses of the same order of magnitude.

Earlier studies on different dose rates have been reported in the literature *(1)*, but unfortunately these are not comparable with ours because the exposure times in the earlier studies were of the order of magnitude of a milli- to a micro-second and the data being compared were obtained not in the same laboratory but in various laboratories. The results of our experiments (Table 12) cannot be extrapolated to exposure times of micro-seconds in duration without making further observations at exposure times between the 2 extremes of micro-seconds and several minutes. However, according to the present investigation the results obtained indicate that, within the range of dose rates studied, dose rate is not a factor in the bactericidal effects of ionizing radiations.

DISCUSSION OF RESULTS

In all, over 1,000 cultures of *E. coli* and *B. thermoacidurans* were prepared and exposed to the 3 different types of radiation mentioned, under the various conditions discussed previously. This large number of samples is mentioned only as justification of the confidence placed in the results.

To our best knowledge, this report presents the results of the first study on the relative bactericidal efficiencies of beta and gamma radia-

TABLE 12

Effect of magnitude of dose rate. E. coli exposed to 3 m.e.v. cathode rays

Run no.	Date (1952)	Number of samples	Approximate dose range ($rep \times 10^{-4}$)	Total energy received: ln n_0/n (average relative values)
Dose rate ca. 600,000-3,000,000 rep_{Si}/min.				
I	March 19	16	1.0- 5	0.78
II	March 20	11	1.0- 5	0.84
III	March 21	12	5.0-20	0.91
IV	March 24	12	5.0-20	0.89
		51 total		
Dose rate ca. 1,500-2,500 rep_{Si}/min.				
V	March 25	3	10.0-50	0.98
VI	March 26	8	0.8- 4	0.76
VII	March 27	2	0.8-	0.65
VIII	March 28	12	0.8- 5	1.00
		25 total		

Statistical analysis

For Runs I-IV:
$\bar{X}_a = 0.89$
$\sigma_a = 0.16$
$n_a = 51$

For Runs V-VIII:
$\bar{X}_b = 0.85$
$\sigma_b = 0.17$
$n_b = 25$

On 95% confidence level:
$\bar{X}_a - \bar{X}_b = 0.04 \pm 0.07$
∴ Difference not significant

tions conducted in the same laboratory by the same personnel, with the use of the same carefully calibrated dosimeters.

Control dosimetric measurements were made in all experiments with the particle accelerator so that any possible variations in accelerator output (momentary transients) that might have occurred would be picked up by the dosimeter ampoules. Because the isotopic source was calibrated by the same dosimeters as used for the particle accelerator and correction was subsequently made for decay, the data obtained are comparable.

Although a few statistically significant differences were noted in the bactericidal effects produced by the different types of radiation, these differences are small percentagewise (only 5 to 15%), and are what one would expect in view of the differences in geometry of the samples.

Table 13 presents in summary form a comparison of the effects on bacteria of gamma rays from the Cobalt-60 source and of X-rays and cathode rays from the Van de Graaff accelerator. These effects are expressed in terms of the mean D_0 values for the different experiments. Analysis of these values indicates the following facts:

(a) In the case of *E. coli* exposed in 3-ml. samples to gamma rays from the Cobalt-60 source and to X-rays of comparable wave-length from the Van de Graaff accelerator, the arithmetical mean D_0 values for the 2 types of radiation agree with each other within about 15%.

(b) Gamma rays from the Cobalt-60 source and cathode rays from the particle accelerator had different relative bactericidal efficiencies, dependent on the thickness and the volume of the samples of *E. coli* used. The relative bactericidal efficiency of cathode rays was apparently lower

when thick samples were used than when thin samples were used. However, when very thin layers of *E. coli* cultures were exposed to cathode rays, D_o values were obtained that were almost identical with those obtained when the ionizing radiations were gamma rays from the Cobalt-60 source or X-rays from the same Van de Graaff accelerator. The theoretical derivation that was made of this effect of the thickness of samples exposed to cathode rays is confirmed by actual experimentation.

(c) In the case of *B. thermoacidurans* exposed to 3 m.e.v. cathode rays produced in the Van de Graaff accelerator and to gamma rays from the Cobalt-60 source, the mean D_o values agree with each other within about 10%. This confirms the conclusion derived from the observations on *E. coli* that the relative bactericidal effectiveness of the 2 sources is essentially the same when thin samples are exposed.

(d) Statistical analyses (Table 14) of the tremendous amount of data obtained confirm the general trends discussed above.

(e) When *E. coli* in ampoules was irradiated by the Cobalt-60 gamma rays, the radiations, in the dose range studied, were less effective in destroying bacteria in 5-ml. samples than in 3-ml. samples. No such effect of sample volume was observed with *B. thermoacidurans* irradiated in similar ampoules under similar conditions. Likewise with dosimeter solutions (ferrous sulfate) exposed in identical ampoules in volumes of 3 and 5 ml., no volume effect was noted. The reason for the volume effect in the case of *E. coli* is not known.

TABLE 13

Summary: comparative effects on bacteria of γ-rays from Cobalt-60 source and X-rays and cathode rays from Van de Graaff accelerator

Organism	Source	Container	Volume of sample	Total no. of samples	Mean D_o ($reps_{as} \times 10^{-3}$)
E. coli	Van de Graaff cathode rays	Ampoules	3 ml.	61	7.48
E. coli	Van de Graaff X-rays	Ampoules	3 ml.	108	5.64
E. coli	Van de Graaff cathode rays	Petri dishes, large	5 ml. (0.081 cm.)	54	5.23
E. coli	Van de Graaff cathode rays	Petri dishes, small	10 ml. (0.51 cm.)	52	10.3
E. coli	Van de Graaff cathode rays	Petri dishes, small	2 ml. (0.1 cm.)	52	6.21
E. coli	Cobalt-60	Ampoules	3 ml.	196	6.45
E. coli	Cobalt-60	Ampoules	5 ml.	184	10.1
B. thermoacidurans	Van de Graaff cathode rays	Ampoules	3 ml.	118	50.1
B. thermoacidurans	Cobalt-60	Ampoules	3 ml.	157	45.6
B. thermoacidurans	Cobalt-60	Ampoules	5 ml.	22	47.2

TABLE 14

Statistical differences between means of D_{37} values obtained in comparative experiments

Data means† being compared		Means, standard deviations, and total numbers of samples						95% confidence limits		99% confidence limits	
a	b	X_a	a	N_a	X_b	b	N_b	$X_a - X_b$	Significance	$X_a - X_b$	Significance
A	C	6.49	1.16	143	6.35	1.97	53	0.14±0.56	No	0.14±0.74	No
B	D	10.1	1.6	118	10.1	2.4	66	0.0 ±0.6	No	0.0 ±0.9	No
A+C	B+D	6.55	1.42	196	10.0	1.9	184	−3.4 ±0.3	Yes	−3.4 ±0.4	Yes
A+C	E	6.55	1.42	196	7.48	1.88	61	−0.93±0.51	Yes	−0.93±0.67	Yes
A+C	F	6.55	1.42	196	5.64	1.64	108	0.91±0.37	Yes	0.91±0.48	Yes
A+C	G	6.55	1.42	196	5.23	1.06	54	1.32±0.34	Yes	1.32±0.45	Yes
A+C	H	6.55	1.42	196	10.3	3.4	52	−3.7 ±0.9	Yes	−3.7 ±1.2	Yes
A+C	I	6.55	1.42	196	6.21	0.76	52	0.34±0.29	Yes	0.34±0.38	No
B+D	I	10.0	1.9	184	6.21	0.76	52	3.8 ±0.3	Yes	3.8 ±0.5	Yes
H	I	10.3	3.4	52	6.21	0.76	52	4.1 ±0.9	Yes	4.1 ±1.2	Yes
G	H	5.23	1.06	54	10.3	3.4	52	−5.1 ±1.0	Yes	−5.1 ±1.3	Yes
E	G	7.48	1.88	61	5.23	1.06	54	2.25±0.55	Yes	2.25±0.72	Yes
E	H	7.48	1.88	61	10.3	3.4	52	−2.8 ±1.0	Yes	−2.8 ±1.4	Yes
E	I	7.48	1.88	61	6.21	0.76	52	1.27±0.52	Yes	1.27±0.67	Yes
G	I	5.23	1.06	54	6.21	0.76	52	−0.98±0.35	Yes	−0.98±0.46	Yes
F	G	5.64	1.64	108	5.23	1.06	54	0.41±0.42	No	0.41±0.55	No
K	L	45.3	5.6	125	46.7	3.2	32	−1.4 ±1.5	No	−1.4 ±1.9	No
L	M	46.7	3.2	32	47.2	2.9	22	−0.5 ±1.6	No	−0.5 ±2.2	No
K+L+M	J	46.2	4.9	179	50.1	7.0	118	−3.9 ±1.5	Yes	−3.9 ±1.9	Yes

Designation	Organism	Source	Volume of culture	Container	Table reference no.
A	*E. coli*	Cobalt-60	3 ml.	Ampoule	12
B	*E. coli*	Cobalt-60	5 ml.	Ampoule	13
C*	*E. coli*	Cobalt-60	3 ml.	Ampoule	14
D*	*E. coli*	Cobalt-60	5 ml.	Ampoule	14
E	*E. coli*	Cathode rays	3 ml.	Ampoule	15
F	*E. coli*	X-rays	3 ml.	Ampoule	16
G	*E. coli*	Cathode rays	5 ml.	Petri dish large	17
H	*E. coli*	Cathode rays	10 ml.	Petri dish small	18
I	*E. coli*	Cathode rays	2 ml.	Petri dish small	18
J	*B. thermoacidurans*	Cathode rays	3 ml.	Ampoule	19
K	*B. thermoacidurans*	Cobalt-60	3 ml.	Ampoule	20
L**	*B. thermoacidurans*	Cobalt-60	3 ml.	Ampoule	21
M**	*B. thermoacidurans*	Cobalt-60	5 ml.	Ampoule	21

† Code designations for "a" and "b."
* C and D samples were irradiated simultaneously.
** L and M samples were irradiated simultaneously.

SUMMARY

The effect of non-uniform ionization density of radiations such as are obtained when cathode rays are absorbed in matter has been derived theoretically, and the theoretical derivation has been substantiated experimentally for both a sporulating and a non-sporulating bacterial organism.

A volume effect was found on exposure of *E. coli* to the Cobalt-60 gamma rays. This volume effect was not found with spores of *B. thermoacidurans*.

With both these test organisms, if the samples were sufficiently thin (0.1 cm. or less), high-energy cathode rays and X-rays from the particle accelerator and high-energy gamma rays from the Cobalt-60 source all had essentially the same relative bactericidal effectiveness, within a very few per cent.

If, however, the thickness of the samples was such that the variation in ionization from top to bottom of the samples was 40%, the relative bactericidal efficiency of the cathode rays was less than that of the gamma rays or the X-rays by a factor of 6:10 approximately. This fact is of importance from the standpoint of practical processing, for in the processing of foods it is unlikely that thin samples will be available for most operations. On the contrary, it is expected that it will be necessary for ionizing radiations to penetrate thick samples. For this reason it would appear that, under conditions of equal absorption of energy, the relative bactericidal effectiveness of high-energy cathode rays would be less than that of gamma rays or of X-rays.

These differences in relative efficiencies observed when bacteria were under study were not noted with chemical and physical dosimeters. The explanation of this is that these dosimeters record the same average dose for equal amounts of energy from both a homogeneous and a non-homogeneous ionization beam.

In the case of cathode rays, therefore, it may be desirable from the standpoint of bactericidal effect to express doses as minimum rather than average doses. Non-uniform ionization effects (overdose) will then come into the throughput calculation as the efficiency factor *(3)*.

Average dose values as obtained from either chemical or physical dosimeters can be converted to minimum dose values by simple calculations (e.g., Eqs. 9 and 10), which can be made if one knows the thickness of the dosimeter solution and the characteristics of the absorber to be sterilized.

The investigation reported herein presents for the first time an intensive bacteriological evaluation of the relative effectiveness of beta and gamma radiations on 2 bacterial organisms under controlled and strictly comparable conditions. Samples were exposed in containers of geometrical and absorber characteristics identical with those of the chemical dosimeters employed. This was possible because dosimeters of the chemical-solution type were used whose efficacy for this particular comparative series of experiments has been confirmed and justified.

In view of the paucity of information available on the subject of the relative bactericidal efficiencies of beta and gamma radiations, this investigation is being continued and extended to other spore-forming organisms in pure cultures and in inoculated food packs.

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Addendum

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LITERATURE CITED

1. Brasch, A., and Huber, W. Ultrashort application time of penetrating electrons: A tool for sterilization and preservation of food in the raw state. *Science*, **105**, 112 (1947).
2. Davison, S., Goldblith, S. A., Proctor, B. E., Karel, M., Kan, B., and Bates, C. J. Dosimetry of a kilocurie Cobalt-60 source. *Nucleonics* (in press, 1953).
3. Goldblith, S. A., and Proctor, B. E. Evaluation of food sterilization efficiency. *Nucleonics*, **10** (9), 28 (1952).
4. Goldblith, S. A., Proctor, B. E., Davison, S., Lang, D. A., Kan, B., Bates, C. J., and Karel, M. Studies in the dosimetry and bactericidal effects of gamma radiations from a Cobalt-60 source. *Radiology*, **60** (5), 732 (1953).
5. Hayner, J. H., and Proctor, B. E. Investigations relating to the possible use of atomic fission products for food sterilization. *Food Technol.*, **7**, 6 (1953).
6. Lea, D. E. *Actions of Radiations on Living Cells.* 1947, Macmillan Co., New York.
7. Manowitz, B. Use of kilocurie radiation sources. *Nucleonics*, **9** (2), 10 (1951).
8. Manowitz, B. Facilities for gamma irradiation. *Nucleonics*, **11** (3), 18 (1953).
9. Proctor, B. E., and Goldblith, S. A. Food processing with ionizing radiations. *Food Technol.*, **5**, 376 (1951).
10. Proctor, B. E., and Goldblith, S. A. Dosimetry research on the kilocurie Cobalt-60 source of the Department of Food Technology of the Massachusetts Institute of Technology. Contract No. AT(30-1)-1164 with the U. S. Atomic Energy Commission, Sept. 1, 1952. NYO 3339.
11. Robinson, D. M. Electrostatic accelerators in nuclear research. *Nucleonics*, **1** (3), 71 (1947).
12. Rosenzweig, W. A dosimetric study of high intensity gamma sources. B.N.L.-1254 (Nov. 1952).

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BACTO-NIACIN ASSAY MEDIUM
BACTO-THIAMIN ASSAY MEDIUM
BACTO-PANTOTHENATE ASSAY MEDIUM
BACTO-B_{12} ASSAY MEDIUM USP
BACTO-CS VITAMIN B_{12} AGAR
BACTO-FOLIC ACID ASSAY MEDIUM
BACTO-PYRIDOXINE ASSAY MEDIUM
BACTO-BIOTIN ASSAY MEDIUM
BACTO-CHOLINE ASSAY MEDIUM
BACTO-CF ASSAY MEDIUM
BACTO-TRYPTOPHANE ASSAY MEDIUM
BACTO-LEUCINE ASSAY MEDIUM
BACTO-METHIONINE ASSAY MEDIUM
BACTO-LYSINE ASSAY MEDIUM
BACTO-ISOLEUCINE ASSAY MEDIUM
BACTO-ARGININE ASSAY MEDIUM
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The method employed in carrying stock cultures of the test organisms and preparing the inoculum for microbiological assay is important. The following media have been developed especially for carrying stock cultures and for preparation of the inoculum:

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Descriptive literature available upon request

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